PLANT SCIENCE FORMULÆ

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A REFERENCE BOOK FOR
PLANT SCIENCE LABORATORIES
(INCLUDING BACTERIOLOGY)

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PREFACE

ONE of the most useful aids to laboratory work in any science is a file, collecting together the items of practical information which experience has taught the worker are needed from day to day. All research workers keep such records of their own personal requirements, but the compilation of a general compendium of information requires more time and opportunity than most people have at their disposal.

Personal experience, as university teachers and directors of student research, has brought home to us the waste of time and effort entailed by the search for methods or formulæ through volume after volume, and the still greater annoyance that may be caused by the ultimate failure to trace the desired information. It was this that led us to the compilation of this book.

The scope of the book ranges widely over the varied fields in which botanical workers are most frequently interested. The guiding principle throughout has been to include receipts of proved and established worth rather than those having more specialised value. Obviously to include every published receipt is impossible, since to follow the plant sciences from Silurian fossils to spectrography would demand, not a book, but a library. Selection, therefore, has been strict, and we have only included special methods in cases where the work concerned might be expected to enter into the course of good advanced students.

In the great majority of cases we have confined ourselves to very brief indications of the methods of use of the receipts given, since we considered that nearly everyone requiring such receipts would already know their general application. Such notes as we have given are, therefore, concerned only with the peculiarities, if any, of the particular receipt.

We have also omitted any statement of the sources of the receipts, except in the case of famous or of very recent methods,

where the name of the discoverer is easily ascertained. Many receipts are traditional, or have passed through so many hands that it would be invidious to attribute them to any individual authority.

A work of this character is inevitably coloured by the idiosyncracies of its compilers, and we plead guilty in advance of many sins of omission, which may appear more or less heinous to other eyes. Also to some, though we hope few, sins of commission. We would in any case esteem it a great favour if those who find this book useful would help us to improve it by their collaboration in communicating new or improved methods, which we will gratefully acknowledge.

Space has been left at the end of each chapter for the insertion by the reader of additional receipts, etc.

The book has been edited by Mr. L. J. F. Brimble and we gladly acknowledge his help in this connexion.

R. C. McLEAN.
W. R. IVIMEY COOK.

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CHAPTER I

INTRODUCTION

IN the provision of the equipment for a botanical laboratory many different reagents are necessary. Many of these must be purchased in a prepared state, and only need dilution for use. On the other hand there are a large number of solutions necessary in botanical technique, which are prepared by the compounding of a number of different substances together. Considering the amount of information which the botany teacher is expected to have at his finger-tips, it cannot be expected that he should attempt to memorize the composition of these various reagents, even those which are restricted to one particular line of work. Thus Levine and Schönlein have collected together some thousands of receipts for making up culture media ; but the average botany teacher is content to make use of a relatively small number of receipts, which will provide him with media on which to grow the common fungi which he is likely to need for class work.

No research worker seems to consider he has carried out his investigation satisfactorily unless he has taken someone else's fixative, stain or medium, and by some means or other modified it. These modifications may in some instances be justified, for, since botanical material varies in structure, so must the fixatives or stains be altered to obtain the optimum results required by an original investigator. The teacher, however, in the majority of instances is more concerned with quick results, provided they are satisfactory.

It is unreasonable to expect that there should be one universal fixative, one staining method or one nutrient agar, with which every type of botanical material will react equally well. Even the photographer knows that he must alter the composition of

his developer according to whether he wishes his negative to show plenty of tone variations or a purely black and white effect. He is dealing with a sensitive surface of known chemical composition, and therefore is not subject to the difficulties of the botanist who is dealing with material of very varied and largely unknown composition.

The first concern therefore in compiling this book has been to collect together formulæ of general, rather than of special application and to give the composition of all those solutions which are most widely used. Many botanical departments have their own modifications of certain standard reagents, modifications which have been found, as a result of experience, to suit their own conditions better than any others. Such modifications are often desirable; but to many, and especially to the school teachers, a single good, reliable receipt is enough to meet the requirements of each occasion. Some text-books and laboratory guides supply an appendix in which a small selection of reagents, recommended in the book, are explained and their preparation described: but in most cases such lists only go part of the way, and a complete set of the receipts required to make up the stock solutions for an average botanical laboratory can only be obtained by consulting a great variety of sources. This is certainly a waste of time, even if the books are available, and the result is that a file or card-index is usually compiled to record the necessary information. More especially is this necessary in the case of less commonly required receipts. In fact it may be said that this book is little more than our own card-index set out in a more convenient form.

It is a noticeable fact that while the universities of Great Britain expend a large amount of energy in training students as teachers in secondary and public schools, they pay very little attention to fitting the student for the practical application of his training in science. As a student he is supplied with many reagents, but he is rarely told anything about how they are made or where the components may be obtained. In these days when new biological laboratories are being opened in secondary schools or where old ones are being expanded or properly equipped for the first time,

it is no infrequent thing for an old student to go to his university teachers and ask, "Where can I get this from?" or "Whom should I go to for that?" or "How do you make so and so?"

It is hoped that in this book the answers to such questions may be found. This is not intended as a book for the library, but a book for the laboratory: a book where the answers to all the little odds-and-ends of problems in the running of a laboratory may be found. If in the course of time it becomes thumbed and its pages covered with stains and acid burns, so much the better, for it will be serving its purpose as a book in which to look things up, as a book meant to be left lying about on the laboratory table, not put away in some safe but relatively inaccessible place.

CHAPTER II

SOLUTIONS FOR FIXING AND PRESERVING MATERIAL

THE number of fixing and preserving ingredients is relatively small. The purpose of a killing agent is that it should do its work quickly, thereby not only bringing the life-process to a sudden end, but also fixing the cells so far as possible in the same condition as they were in during life. For this purpose two things are necessary: first that the fixative shall have a hardening effect upon the cells, and second that it shall penetrate into the tissues as quickly as possible. The rate at which fixatives penetrate into plant tissue is relatively slow, as the accompanying table will indicate.

PENETRATION OF FIXATIVES

Substance	Distance of penetration in millimetres in given time			
	1 hr.	4 hr.	12 hr.	26 hr.
1% Picric acid	0·5	1·0	1·5	1·5
½% Osmic acid	0·25	0·75	1·0	1·0
1% Chromic acid	0·5	1·5	2·5	4·0
Flemming's solution	1·0	2·0	4·5	5·0
10% Formalin	0·5	2·0	2·5	5·0
70% Alcohol	0·5	1·25	2·5	8·0
96% Alcohol	1·0	1·75	3·5	8·0
5% Acetic acid	1·0	2·5	4·0	8·0
70% Alc., 5% Form., 5% Acetic	2·0	3·0	6·0	8·0

(From Krause, *Encyclopädie der mikroscopischen Technik.*)

From this table it will be seen that the principal agents used in fixation are alcohol, chromic acid, acetic acid, osmic acid, picric acid and formalin. To these may be added chloroform, potassium dichromate, potassium iodide, copper acetate, formic acid, nitric acid and mercuric chloride.

Many of these solutions damage the tissues if not washed out after they have done their work. Normally those which are made up in water are washed out in water, while those made up in alcohol are washed out with alcohol of the same strength. The material may then be stored in various fluids, of which alcohol and formalin form the chief constituents. A table for the preparation of dilutions of alcohol is given on p. 150.

Fixatives therefore fall into a number of main groups according to their ingredients. The selection of a fixative must depend on the type of material and the conditions under which the collection is being made. If away from home it may be necessary to retain the material in the fixative for some time, in which case certain tissue-destroying fixatives must be avoided. Similarly some fixatives, which will give excellent results with the harder parts of woody plants, would be far too drastic in their action for use on delicate algæ. To obtain a universal fixative is probably impossible, though some have a far more general application than others. Some are relatively cheap to make up, and this may be important when large quantities of material are required.

Despite the trouble involved, it cannot be too strongly emphasized that all botanical material, whether for anatomical investigation by a junior class, or for cytological research work, should be cut up and properly fixed, as a matter of routine, immediately it is collected, and not be left for hours or days in a vasculum before finally being plunged whole into a bottle of alcohol.

FIXATIVES AND PRESERVATIVES

Form-Alcohol (Chicago Formula) (1)

Alcohol 70 per cent - - -	100 c.c.
Formalin - - - - -	6 c.c.

Material may be fixed and stored in this fluid indefinitely.

Form-Alcohol (Lynds Jones Formula) (2)

Alcohol 70 per cent - - -	100 c.c.
Formalin - - - - -	2 c.c.

May be used either as a fixative or for storing material.

General Fixative (King's Formula) (3)

Formalin 5 per cent	100 c.c.
Alcohol 95 per cent	150 c.c.
Glycerine	50 c.c.

Universal Fixative (Rawlin's Formula) (4)

Alcohol 50 per cent	100 c.c.
Formalin	6·5 c.c.
Glacial acetic acid	2·5 c.c.

Acetic Alcohol Fixative (Farmer's Formula)

Glacial acetic acid	1 part
Absolute alcohol	3 parts

Wash well in strong alcohol for 15 minutes after fixation and store material in 70 per cent alcohol.

Carnoy's Fixative

Absolute alcohol	2 parts
Chloroform	3 parts
Glacial acetic acid	1 part

Fix for 10–15 minutes and wash in 85 per cent alcohol, in which the material may be stored.

Osmic Acid Fixative

Osmic acid	1 gm.
Distilled water	100 c.c.

The solution must be kept in the dark. It is best prepared by breaking the clean tube in which it is bought, in the necessary distilled water. The water may be placed in the bottle, and the tube slipped in and broken by shaking. If exposed to the light metallic osmium is precipitated and the solution becomes useless. This may be avoided by dissolving in 1 per cent chromic acid instead of in distilled water.

Chromic Acid Fixative (1)

Chromic acid	10 gm.
Glacial acetic acid	10 gm.
Water	1,000 c.c.

Fix for 24 hours and wash in water.

Various other strengths of this fixative may be made up according to the nature of the material. The above is suitable for most cytological material; but a weaker solution, say, chromic acid 3 gm., glacial acetic acid 7 gm., water 1,000 c.c., may sometimes be used.

Chromo-Acetic Fixative (2)

Strong	Chromic acid 1 per cent	140 c.c.
	Glacial acetic acid	1 c.c.
Weak	Chromic acid 1 per cent	140 c.c.
	Glacial acetic acid	1 c.c.
	Water	60 c.c.

Schaffner's Chromo-Acetic Fixative (3)

Chromic acid	0·3 gm
Glacial acetic acid	0·7 c.c.
Water	99·0 c.c.

A useful modification of the standard formula.

Benda's Fixative (4)

Chromic acid 1 per cent	16 c.c.
Osmic acid 2 per cent	4 c.c.
Glacial acetic acid	2 drops

Wash in water and grade through alcohol as for normal chromo-acetic fixatives.

Licent's Fixative (5)

Chromic acid 1 per cent	80 c.c.
Glacial acetic acid	5 c.c.
Formalin	15 c.c.

Chicago Fixative (6)

Chromic acid	1 gm.
Glacial acetic acid	2 c.c.
Osmic acid 1 per cent	6–8 c.c
Water	90 c.c.

A very useful general fixative.

Chromo-Acetic Fixative for Marine Algæ (7)

Chromic acid	1·0 gm.
Glacial acetic acid	0·4 c.c.
Sea water	400·0 c.c.

Material must be washed in sea water

Navaschin's Fixative

Solution *A*	Chromic acid	1·5 gm.
	Glacial acetic acid	10·0 c.c.
	Distilled water	90·0 c.c.
Solution *B*	Formalin	40·0 c.c.
	Distilled water	60·0 c.c.

Mix equal quantities of solutions *A* and *B* immediately before use. A good fixative for mitotic figures in ferns and higher plants.

Rabl's Fluid

Chromic acid 10 per cent	7 c.c.
Formic acid (specific gravity 1·2)	5 drops
Water	200 c.c.

May be used for fixing nuclei and mitotic figures.

Flemming's Fixative (1)

Strong	Chromic acid 1 per cent	75 c.c.
	Glacial acetic acid	5 c.c.
	Osmic acid 2 per cent	20 c.c.
Weak	Chromic acid 1 per cent	25 c.c.
	Acetic acid 1 per cent	10 c.c.
	Osmic acid 1 per cent	10 c.c.
	Water	50 c.c.

Flemming's Solution (Newton's Modification) (2)

Chromic acid 1 per cent	14 c.c.
Osmic acid 2 per cent	4 c.c.
Glacial acetic acid	0·5 c.c.
Water	12 c.c.

Fix for about 6 hours. Wash for 24 hours in water.

N.K.L. Fixative (Navaschin-Karpechenko-Langlet Formula)

Solution *A*	Chromic acid	1 gm.
	Acetic acid	10 c.c.
	Water	65 c.c.
Solution *B*	Formalin	40 c.c.
	Water	35 c.c.

Use in equal parts and keep cold. It will frequently be found better to use at half strength for a general fixative. Material may be left in the fixative for some time.

La Cour Fixatives

2 *BD*	Chromic acid 1 per cent	100 c.c.
	Potassium dichromate 1 per cent	100 c.c.
	Acetic acid 5 per cent	30 c.c.
	Osmic acid 2 per cent	30 c.c.
	Saponin	0·1 gm.
2 *BE*	Chromic acid 1 per cent	90 c.c.
	Acetic acid 5 per cent	10 c.c.
	Osmic acid 2 per cent	15 c.c.
	Potassium dichromate	1 gm.
	Saponin	0·05 gm.

Uranium trioxide may replace the osmic acid. Bleach with hydrogen peroxide (1 : 4 of 80 per cent alcohol). Stain with iodine-gentian violet.

Smith's Fixatives for Pollen Mother Cells

S_1	Chromic acid 1 per cent	110 c.c.
	Osmic acid 2 per cent	35 c.c.
	Acetic acid 5 per cent	25 c.c.
	Potassium dichromate	0·5 gm.
	Saponin	0·05 gm.
	Distilled water	50 c.c.
S_2	Chromic acid 1 per cent	75 c.c.
	Osmic acid 2 per cent	25 c.c.
	Acetic acid 5 per cent	12·5 c.c.

Potassium dichromate	1 gm.
Saponin	0·05 gm.
Distilled water	46 c.c.

Gilson's Fixative

Alcohol 95 per cent	42·0 c.c.
Glacial acetic acid	18·0 c.c.
Nitric acid	2·0 c.c.
Mercuric chloride sat. aq.	11·0 c.c.
Water	60·0 c.c.

Wash with 70 per cent alcohol after fixing for about 10 minutes.

Dichromate Fixative (Champy Formula)

Potassium dichromate 3 per cent	7 parts
Chromic acid 1 per cent	7 parts
Osmic acid 2 per cent	1 part

Fix for 6–24 hours. Wash in running water for about the same time. This fixative is good for cytoplasmic structures.

Bensley's Fixative

Mercuric chloride 2·5 per cent in water	4 parts
Osmic acid 2 per cent	1 part

This fixative can be used for mitochondria.

Mercury-Alcohol Fixative

Mercuric chloride	10 gm.
Nitric acid	5 c.c.
Glacial acetic acid	2 c.c.
Alcohol 95 per cent	30 c.c.
Water	270 c.c.

Fix for 1 hour and wash in 50 per cent alcohol. Store in 70 per cent alcohol.

Mercury-Picric Fixative

Mercuric chloride	5 gm.
Glacial acetic acid	5 c.c.
Picric acid (sat. sol. in 30 per cent alc.)	100 c.c.

Fix for 1 hour and wash in 50 per cent alcohol. Store in 70 per cent alcohol.

Mercury-Acetic Fixative

Mercuric chloride	3 gm.
Glacial acetic acid	3 c.c.
Alcohol 95 per cent	100 c.c.

Fix for 1 hour and wash in 95 per cent alcohol. Store material in 70 per cent alcohol.

Zenker's Fixative

Potassium dichromate	2·5 gm.
Mercuric chloride	5·0 gm.
Glacial acetic acid	5·0 gm.
Water	100 c.c.

When mixed add 10 c.c. of formalin. Fix for 24 hours, wash in water, and treat sections with iodine before staining.

Picric Acid Fixative

Picric acid	1 gm.
Water or alcohol 70 per cent	100 c.c.

This may be used cold for 1–24 hours, or hot (85° C.) for 5–10 minutes. Washing must be continued until the yellow colour of the picric acid disappears.

Bouin's Fluid (1)

Picric acid (sat. aq. sol.)	75 c.c.
Formalin	25 c.c.
Glacial acetic acid	5 c.c.

The fixative should be allowed to act for 24 hours. It may be left longer. Wash in water and grade up through alcohol. Store in 70 per cent alcohol.

Bouin's Fluid (Allen's modification) (2)

Picric acid (sat. aq. sol.)	75 c.c.
Formalin	25 c.c.
Glacial acetic acid	5 c.c.

Add at time of fixing :

Urea - - - - - -	2 gm.
Chromic acid - - - -	1·5 gm.

Heat fixative to 38° C. and fix for 1 hour. Cool to room temperature and dehydrate by dropping one drop of absolute alcohol per second into the fixative so that material has reached 75 per cent alcohol in 1 hour. Wash out the picric acid with 75 per cent alcohol, containing a few drops of lithium carbonate. Shake frequently until the yellow colour has disappeared.

Picro-Sulphuric Acid Fixative (Kleinenberg)

Picric acid (sat. aq. sol.) - -	100 c.c.
Sulphuric acid - - - -	2 c.c.
Distilled water - - - -	300 c.c.

This fixative is more generally useful for zoological than for botanical work, but may be found useful for anatomical work. The solution should be filtered before use.

Fixative for Protoplasmic Connections (Meyer)

Sulphuric acid - - - -	2 c.c.
Water - - - - - -	100 c.c.
Picric acid to sat. = about 0·25 per cent	

Fix for 2 hours, then wash out in 70 per cent alcohol. Place fixed material in sulphuric acid of strength between 2 and 50 per cent for at least half an hour before staining. Some material, for example, moss leaves, needs up to 24 hours in acid of 50 per cent strength or over.

Merkel's Fluid

Chromic acid 1 per cent - -	25 c.c.
Platinic chloride 1 per cent - -	25 c.c.
Water - - - - - -	150 c.c.

Recommended for nuclear division in higher plants. It is probably no better than chromo-acetic fixatives.

Hermann's Fixative

Platinic chloride 1 per cent - -	15 parts
Glacial acetic acid - - -	1 part
Osmic acid 2 per cent - - -	2–4 parts

This is a modification of Flemming's solution, and it is used for similar purposes and in a similar manner.

Iodine Fixative

A saturated solution of iodine in a saturated solution of potassium iodide in water will be found a useful fixative for unicellular and colonial algæ. Fix for 12–24 hours, wash in water, or, if starch is present, use a 0·5 per cent solution of tannic acid in water.

Oxyquinoline Sulphate (Chinosol) as Preservative

Plant material may be easily and completely preserved in the field by packing in containers, preferably of glass or earthenware, covering with water and adding enough chinosol to make a solution of strength up to 1 per cent. The chinosol is in powder form, and the procedure has great advantages over formalin or alcohol, especially for travellers.

CHAPTER III

DEHYDRATING, CLEARING AND MOUNTING MEDIA

IN the preparation of microscopic slides many solutions have been devised, though so far as dehydration is concerned only alcohol need be mentioned here. Various grades of alcohol are required, and should be diluted from the stock. For most botanical purposes the spirit supplied as " Industrial Methylated Spirit, 74 O.P.", is the most satisfactory. It should be specified as Toilet Quality ". It is free from water and from colouring matter, and contains about 99 per cent alcohol. Tables are given on p. 150 for the dilution of this to suitable strengths. Only for special purposes is absolute alcohol necessary, and this need not be supplied for general class purposes.

The methylated spirit (about 95 per cent alcohol) usually sold does not make clear mixtures with water, or else it contains a little gentian violet colouring. The latter may be removed by filtering through animal charcoal (bone charcoal). Better grades can, however, be obtained, and where a class is to be supplied it is worth while to apply to the local excise officer for a permit to purchase the industrial grade of spirit, which costs about 2*s.* 6*d.* a gallon. The excise authorities will give directions where the spirit is to be purchased. The dealers do not supply less than 10 gallons at a time.

Single workers or small classes will find it better to use either *iso*-propyl alcohol or *n*-butyl alcohol in place of the expensive ethyl alcohol (='alcohol' in the usual sense). These alcohols may be purchased without licence, and can be used for most microscopical purposes.

Before a preparation is mounted in balsam or other resinous medium, it must be dehydrated, either with absolute ethyl alcohol or with a cheaper substitute, such as *iso*-propyl alcohol or *n*-butyl

alcohol or even acetone. With care the industrial spirit mentioned above may often prove sufficient, but it is not so reliable as the others. Transference from the dehydrant agent to the balsam is made through a clearing agent.

Clearing agents render the object transparent, and are most of them ethereal oils or coal tar oils. Xylol (xylene) is probably the most convenient and generally used clearing agent, but cedarwood oil, clove oil, bergamot oil, turpentine, chloroform, carbolic acid and benzol all have their uses. These substances will mix only with a small proportion of water, and the selection of a clearing agent depends to some extent, therefore, upon the degree of dehydration which it is proposed to employ. The following gives the lowest percentage of alcohol with which some of the common clearing agents will mix without precipitation :

Bergamot oil	90 per cent alcohol
Clove oil	90 per cent alcohol
Turpentine	90 per cent alcohol
Cedar-wood oil	92 per cent alcohol
Xylol	96 per cent alcohol

The desirability of being able to transfer sections directly from the dehydrant to a mountant and thereby eliminate the clearing agent has been felt for a number of years, and various proprietary articles have been put on the market to meet this end. Euparal, for example, may be used in place of balsam, and sections transferred into it directly from absolute alcohol or from 90 per cent alcohol.

Other substances have also been utilized for clearing or mounting, and the accompanying list may be found useful.

Refractive Indices of Mounting Media

Water	1·33
Glycerine jelly	1·45
Liquid paraffin	1·47
Glycerine	1·47
Euparal	1·48
Gum dammar	1·52

Cedar oil (thin)	1·52
Xylol-balsam	1·52
Venetian turpentine	1·54
Canada balsam, filtered	1·54
Clove oil	1·57
Styrax	1·60
Monobromo-naphthalene	1·66
Hyrax	1·80
Realgar	2·55

Refractive Indices of Typical Glasses

Fluor-spar	1·433
Crown glass (various)	1·5–1·6
Flint glass (various)	1·54–1·8

Canada Balsam

Although numerous mounting media have been employed in botanical work, Canada balsam is still the most generally used substance for permanent preparations.

Solutions may be made or bought. The dried resin may be dissolved either in xylol or benzyl alcohol. The latter hardens more quickly, and is generally better for microscopic work.

Various other solvents are used, such as benzol and turpentine; but they have little advantage over the above. The pure filtered resin may be used, though it hardens slowly, or it may be used for dissolving instead of the dried substance.

Glycerine Jelly

Gelatine	1 part
Glycerine	7 parts
Water	6 parts

Warm the gelatine in the water for about 2 hours and then add the glycerine. Warm and stir for 15 minutes, adding 1 per cent phenol as a preservative.

Steimetz' Fluid (See p. 71)

Lacto-phenol (Amann)

Lactic acid	100 c.c.
Phenol	100 gm.
Glycerine	100 c.c.
Water	100 c.c.

Dissolve the phenol in water without heat, to prevent oxidation. Then add the glycerine and lactic acid.

The disadvantage of the low refractive index may be obviated either by adding a dye to the solution, for example, saturated aqueous picric acid instead of water (or see p. 39), or by dissolving in it a substance which will raise the refractive index (see p. 18).

Eycleshymer's Clearing Fluid

Bergamot oil	50 c.c.
Cedar-wood oil	50 c.c.
Phenol	50 c.c.

This mixture clears readily from 95 per cent alcohol, and is consequently particularly useful for celloidin sections. It is not suitable for use prior to infiltration with paraffin.

Glycerine

Plain glycerine is the most generally used examining and mounting fluid for temporary preparations. It should be diluted with water to about 30 per cent strength. It causes some shrinkage of delicate objects.

Universal Mountant Fluid (McLean)

Copper acetate	0·50 gm.
Glacial acetic acid	0·75 c.c.
Cane sugar	1·5 gm.
Thymol	1 small crystal
Water	100 c.c.

To this mixture add sheet gelatine 1·15 gm., which is dissolved with the smallest possible amount of warming over a water-bath. The gelatine must be carefully weighed, as the success largely

depends on this. The medium should be only just liquid and quite thick.

Diethylene Glycol Examining Fluid (McLean)

Diethylene glycol	80 c.c.
Water	20 c.c.

This has a lower refractive index than glycerine. It causes relatively little shrinkage, and is therefore useful for green algæ. If enough copper acetate is dissolved in the fluid to give it a pale blue tinge, the colour of the chlorophyll will be preserved. Evaporates very slowly and may be ringed or luted.

Gum Dammar

Gum dammar	100 gm.
Xylol	100 c.c.

This is a convenient formula, but is subject to modifications. An old formula still used sometimes is :

Solution *A*	Gum dammar	100 gm.
	Turpentine oil	200 c.c.
Solution *B*	Gum mastic	50 gm.
	Chloroform	200 c.c.

Mix the two solutions for use.

Farrants' Medium

Arsenious acid	1 gm.
Water	200 c.c.
Gum acacia	130 gm.
Glycerine	100 c.c.

Dissolve in this order and filter. This medium is recommended chiefly because it dries at the edges of the mount and fixes the cover-glass.

Mounting Media of High Refractive Index

Saturated solution in glycerine :

Zinc iodate	R.I. 1·560
Cadmium chloride	R.I. 1·504
Zinc phenol sulphonate	R.I. 1·501
Chloral hydrate	R.I. 1·510

High Refractive Liquid (Stephenson)

Mercuric iodide
Potassium iodide
Excess of each in water

Refractive index 1·68. If glycerine is added in place of part of the water the refractive index may be raised as high as 1·80. It may be diluted as desired, with a corresponding reduction in the refractive index. Not suitable for permanent mounting.

High Refractive Mounting Medium (Amann)

Mercuric iodide - - - -	65 gm.
Potassium iodide - - - -	50 gm.
Pure glycerine, hot - - - -	25 c.c.

The refractive index of this solution may rise as high as 1·78–1·80. Slides mounted in it may be ringed with amber copal or with dammar to which 2 per cent of boiled linseed oil has been added. The solution is dangerous to oil-immersion lens mountings.

High Refractive Index Mounting Media

Styrax, a natural balsam, which should be dissolved either in benzol or in a mixture of equal parts of absolute alcohol and ether, to the consistency of olive oil. Refractive index about 1·58. The balsam is obtained from *Liquidambar orientalis.* The original samples had a refractive index of 1·59. Recent American material has a refractive index of 1·63.

Monobromide of naphthalene, an oily liquid with a refractive index of 1·658, which is used principally for diatoms ; considered not to keep well.

Realgar (arsenic disulphide). Fuse pieces on the slide. Forms a glassy medium with a refractive index of more than 2·0. Only suitable for hard objects like diatoms.

Barff's boro-glyceride, a pharmaceutical product with a refractive index of about 2·0.

Ringer's Fluid (for living cells) (See p. 86)

Physiological Saline (Locke)

Sodium chloride - - -	0·9 gm.
Potassium chloride - - -	0·01 gm.
Calcium chloride - - -	0·02 gm.
Water - -	100·0 c.c.

An ionically balanced solution, for the examination of living objects.

Mounting Living Objects for Dark-ground Illumination (Shmammi)

Mix the organisms on the slide with either 3 per cent bouillon agar or a mixture of horse serum one part, and 5 per cent glucose agar two parts. Lay on a cover-slip and allow to set. Use large covers. Slides may be ringed with wax.

Organisms retain their vitality and move slowly. Flagella and internal structure may be seen well.

Pollen Preparations

Fresh pollen may be mounted in glycerine jelly tinged with methyl green or erythrosine. Pollen in the air-dry state is better mounted in liquid paraffin.

FINISHING MICROSCOPE PREPARATIONS

Shellac Varnish for Ringing Slides

Melt 2 oz. orange shellac in a double-walled pot, such as a gluepot, with enough alcohol to cover it. When dissolved, filter through warm muslin. The addition of 2 per cent castor oil will prevent the varnish from cracking.

Sealing Medium for Wet Preparations

Canada balsam - - - -	5 parts
Gum mastic - - - - -	4 parts
Powdered resin - - - -	3 parts
Anhydrous lanoline - - -	4 parts

The substances are melted together in a metal container and stirred with a glass rod. The resulting medium is a transparent brown. Before applying the medium, the edges of the cover-glass

should be quite dry, and a thin coat of glycerine jelly may be applied, if necessary, to prevent the sealing mixture from running under the cover-glass.

Gum Dammar

Dissolve as much gum dammar as possible in 50 c.c. xylol. The solution may be thinned with more xylol. The varnish will dry rapidly, especially if warmed on a hot plate for an hour.

Gold Size (Beale)

Melt 24 parts of linseed oil with 1 part of red lead, $\frac{1}{3}$ part of umber. Boil for 3 hours. Pour off the clear liquid and mix with equal parts of white lead and yellow ochre and boil again. Pour off the clear liquid for use.

Marine Glue

Dissolve equal parts of shellac and india-rubber in mineral naphtha by heating thoroughly. It may be rendered thinner by the addition of either naphtha or a solution of potassium hydroxide. Marine glue is unaffected by immersion oil.

Sealing Cover-glasses

Harden Venetian turpentine by heating for 2–3 days on a sand bath. Use a hot wire for sealing round the glass.

Wax Luting Mixture for Wet Mounts

Melt together equal parts of paraffin wax and shreds of old rubber tubing. It should set solid when cold, and is applied with a hot wire.

Krönig's Wax

Melt bees-wax 2 parts and add colophonium (resin) 7–9 parts. Heat until all the resin is well incorporated. Apply with a hot wire.

Labelling Slides during Preparation

The end of the slide may be painted with transparent 'Luc', which is a cold lacquer supplied for domestic purposes. On the dry surface details may be written in indian ink.

PLANT SCIENCE FORMULÆ

CHAPTER IV

STAINS AND STAINING METHODS

FEW branches of botanical technique have in recent years become so complex as that of staining. After the war of 1914–18, when the manufacture of aniline dyes became widespread instead of being an almost entirely German monopoly, many new dyes were introduced to botanical work. Unfortunately, makers and suppliers sold their products under varied names, and many of the aniline dyes are now known under several different names. Moreover the quality of the dyes varied, as did also their solubility. In 1923 a Committee on the Standardisation of Biological Stains was set up, which published a list of biological stains giving all the common synonyms. A list of the more common stains with their numbers and synonyms in accordance with the Committee's list is given on p. 50. At the same time it is felt undesirable in this book to alter the names of the dyes in accordance with this new nomenclature. Dahlia, cotton blue and fuchsine are well-known names, and if replaced by iodine violet, soluble blue and magenta would not be readily recognized by many using the book. On the other hand, many suppliers now list their stains under the new names and do not always give the synonyms.

For botanical work the aniline dyes have almost entirely replaced the vegetable decoctions used by the older microscopists. With the exception of the hæmatoxylins and the carmines, nearly all the botanical stains are aniline dyes. These dyes were originally used for dyeing fabrics and were often impure. Only the pure dyes, bought from a reputable maker, should be used for botanical work.

Stains are classed as either acid or basic, and may be either general or specific according to whether they stain all parts equally or stain some elements more readily than others. Stains

may show a great affinity for the nucleus, for example most basic stains, in which case they are called nuclear stains, or for the cytoplasm, when they are termed plasma stains. Most acid stains are in this latter class.

Only the more common stains are mentioned here, together with a few special methods, recommended for certain special types of organisms. As has been stated already, endless variations of stain combinations and staining schedules have been devised, some rapid and some extremely tedious. It is entirely outside the scope of this book to enter fully into these matters, and only a few methods have been included which seemed to the authors to have some commonly useful application in general botanical work. For a fuller treatment of the uses of stains and the compilation of staining schedules the reader is referred to one of the several excellent existing works, such as Chamberlain's *Methods in Plant Histology* or Lee's *The Microtomist's Vademecum.*

Ehrlich's Hæmatoxylin (1)

Hæmatoxylin	1 gm.
Glacial acetic acid	5 c.c.
Glycerine	50 c.c.
Absolute alcohol	50 c.c.
Distilled water	50 c.c.
Alum in excess.	

Keep in a dark place until the colour becomes red. If well stoppered it will keep indefinitely. May be used in the same way as Delafield's formula.

Delafield's Hæmatoxylin (2)

To 100 c.c. of a saturated solution of ammonia alum add drop by drop a solution of 1 gm. of hæmatoxylin, dissolved in 6 c.c. of absolute alcohol.

Expose to light and air for a week.

Add 25 c.c. of glycerine and 24 c.c. of methyl alcohol.

Filter and allow to stand until sufficiently dark. Filter again and keep tightly stoppered.

The solution should stand for about 2 months before use.

A good general stain for non-lignified tissue.

Boehmer's Hæmatoxylin (3)

Solution *A*	Hæmatoxylin - -	1 gm.
	Absolute alcohol -	12 c.c.
Solution *B*	Alum - - -	1 gm.
	Distilled water - -	240 c.c.

Allow solution *A* to ripen for two months. For use add 10 drops of solution *A* to 10 c.c. of solution *B*. Stain 10–15 minutes.

Heidenhain's Hæmatoxylin (4)

Solution *A* 2 per cent solution of ferric ammonium alum in water.

Solution *B* 0·5 per cent hæmatoxylin in distilled water.

The hæmatoxylin crystals will take about 10 days to dissolve in the water. The stain reaches its greatest efficiency in about 6 weeks. It deteriorates after about 3 months.

A stain made by dissolving the hæmatoxylin in a little strong alcohol and then diluting with water, to speed up the process of maturing, is not so good.

Iron Alum Hæmatoxylin (Benda) (5)

Mordant	Ferrous sulphate	8 gm.
	Sulphuric acid	1·5 c.c.
	Nitric acid -	1·8 c.c.
	Water - -	40 c.c.
Stain	Hæmatoxylin	1 gm.
	Water - -	100 c.c.
Differentiator	Acetic acid -	30 c.c.
	Water - -	100 c.c.

Mordant for 24 hours, wash in water, stain for 12 hours and differentiate with the acetic acid solution.

Iron Hæmatoxylin (Combined stain and mordant) (6)

Solution *A* Hæmatoxylin 0·5 per cent in 95 per cent alcohol.

Solution *B*	Ferric chloride 30 per cent aq.	4 c.c.
	Hydrochloric acid	1 c.c.
	Water to	100 c.c.

Use equal parts of *A* and *B*. Bring sections up to 95 per cent alcohol and stain for 5–15 minutes; wash in water and counter stain if desired.

Mayer's Hæm-Alum Stain (7)

1 gm. hæmatein is dissolved by heat in 50 c.c. of 95 per cent alcohol and added to a solution of 50 gm. of alum in 1,000 c.c. of distilled water. Allow the mixture to cool and settle. Filter. Add one crystal of thymol to prevent development of moulds. This mixture may be used as soon as made up, and it keeps indefinitely.

Borax Carmine (1)

Concentrated solution of carmine (2–3 per cent) in 4 per cent borax is prepared by boiling for about half an hour. Dilute with an equal volume of 70 per cent alcohol. Allow to stand for 24 hours. Filter. When material is completely stained put into 70 per cent alcohol, acidulated with 4–6 drops of strong hydrochloric acid per 100 c.c. Wash in neutral alcohol. Objects should be left in the acidulated alcohol until they acquire a bright, transparent appearance.

Grenacher's Borax Carmine Stain (2)

Carmine	3 gm.
Borax	4 gm.
Distilled water	100 c.c.

Dissolve the borax in water and add the carmine, which quickly dissolves with heat. Add 100 c.c. 70 per cent alcohol and filter.

Alum Carmine Stain (3)

Ammonia alum	4 gm.
Carmine	1 gm.
Water	100 c.c.

Boil the aqueous solution of the alum with the carmine for 20 minutes. Filter and allow to cool.

Stain for about 12 hours and the material will not be over-stained.

Magnesia Carmine (Mayer's) (4)

Carmine	2 gm.
Magnesium hydroxide 2 per cent	100 c.c.

Boil the carmine in the magnesium hydroxide solution for 5 minutes, cool, filter and add 3 drops of formalin.

Picro-Carmine (Ranvier's) (5)

Carmine	5 gm.
Ammonium hydroxide 2 per cent	100 c.c.
Picric acid (sat. aq. sol.)	100 c.c.

Mix the ammonium hydroxide and the picric acid and add the carmine. Evaporate to one-fifth volume, filter when cold. Evaporate to dryness. Take up the residue in water to make a 1 per cent solution.

The above solution may also be prepared with the omission of the picric acid.

Hydrochloric Acid Carmine (Mayer's) (6)

Carmine	4 gm.
Hydrochloric acid	15 drops
Alcohol 85 per cent	95 c.c.
Water	15 c.c.

Boil the carmine in the acid and water until dissolved. Cool and add the alcohol. Neutralize to point of precipitation with ammonium hydroxide, filter. If necessary to dilute, add more alcohol.

Mayer's Carmalum Stain (7)

Carminic acid	1 gm.
Alum	10 gm.
Distilled water	200 c.c.

Dissolve the alum in water with heat, and then add the carminic acid. Add one crystal of thymol to prevent moulds. Decant or filter the solution.

This stain may be allowed to act almost indefinitely as it is unlikely to overstain. Good for algæ.

Belling's Iron Aceto-carmine for Smear Preparations

Heat a 45 per cent solution of acetic acid to boiling, with excess of powdered carmine. Cool and filter.

Add a trace of ferric hydrate dissolved in 45 per cent acetic acid, until the liquid turns bluish-red, but *not* until a precipitate forms. Then dilute with an equal amount of plain aceto-carmine. Dilute further, if necessary, with 45 per cent acetic acid. Heat smear preparations under a cover-glass 4–5 times in the stain, but not to boiling. Place the preparation in a Petri dish with 10 per cent acetic acid. When the cover-glass has separated from the slide, place both in a mixture of absolute alcohol 1 part: acetic acid 1 part. Transfer through mixtures of 3 : 1 and 9 : 1 alcohol-acetic acid, rising to pure alcohol and then to xylol. Remount the cover-glass on the slide with balsam.

The stain lasts only a few days, but is convenient for counting chromosomes in smear preparations of pollen mother-cells. (See p. 48.)

Alum Cochineal Stain

Powdered cochineal - - -	50 gm.
Alum - - - - - -	5 gm.
Water - - - - - -	500 c.c.

Dissolve the alum in water, add the cochineal and boil. Evaporate the liquid to two-thirds of its volume and filter. Add a few drops of phenol to prevent moulds.

General Formula for Aniline-mordanted Dye Solutions

Make a 3 per cent solution of aniline oil in distilled water. Shake well and frequently for a day. Add enough alcohol to bring the whole up to 20 per cent alcohol and add 1 gm. of the stain.

Recommended Stain Combinations for Histological Work

Delafield's hæmatoxylin and safranine.
Basic fuchsine and light green.
Safranine and picric aniline blue.
Gentian violet and Bismarck brown.
Gentian violet and erythrosine.

Recommended Stain Combinations for Cytological Work

Heidenhain's hæmatoxylin and orange *G*.
Ehrlich hæmatoxylin and erythrosine.
Acid fuchsine and methyl green.
Feulgen and light green.
Newton's gentian-violet-iodine and picric acid.

Safranine Stain (1)

Safranine		1 gm.
Alcohol 95 per cent		50 c.c.
Water		50 c.c.

or

Solution *A*	Alcohol-soluble safranine	1 gm.
	Absolute alcohol	100 c.c.
Solution *B*	Water-soluble safranine	1 gm.
	Distilled water	100 c.c.

Mix solutions *A* and *B* in equal proportions.

Safranine (Kraus' Stain) (2)

Safranine 2 per cent in 50 per cent alcohol; 2–24 hours.
Wash in water. Dehydrate.
Wash in a mixture of 20 c.c. absolute alcohol and 80 c.c. xylol.
Counterstain in the following mixture: gentian violet 2 per cent in clove oil; absolute alcohol 2 per cent; and xylol 96 per cent.
Wash in xylol and mount.

Breinl's Triple Stain

Mordant in equal parts of:

1. Saturated solution of iodine in 80 per cent alcohol.
2. Saturated solution of potassium iodide in 80 per cent alcohol.

Stain first for 2 hours in a mixture of equal quantities of saturated alcoholic and aqueous safranine, to which a few drops of aniline oil have been added. Wash in water. Stain for 10 minutes in polychrome methylene blue, prepared by dissolving 0·5 gm. sodium carbonate and 7 gm. polychrome methylene blue in 100 c.c. water. Wash, and finally stain for 10 minutes in a solution of orange tannin in alcohol. Wash in absolute alcohol and differentiate in aniline oil.

Acid Fuchsine Stain

Acid fuchsine	1 gm.
Water	100 c.c.

This stain is good when combined with methyl green. The combination may be used for wood sections. Acid fuchsine has a special affinity for leucoplasts. Stain sections for about 1 hour if possible, and differentiate in a saturated solution of picric acid in 70 per cent alcohol.

Basic Fuchsine Stain

Basic fuchsine	1 gm.
Alcohol 95 per cent	100 c.c.
Water	100 c.c.

Useful for staining vascular systems in higher plants.

Leuco-Basic Fuchsine (for the Feulgen Stain. Coleman)

Basic fuchsine 0·2 per cent	200 c.c.
Potassium metabisulphite	2 gm.
N./1 hydrochloric acid	10 c.c.

Allow the mixture to bleach for 24 hours, then add 0·5 gm. 'Norite'. Shake for a minute and rapidly filter through coarse paper. Alternatively, filter through animal charcoal. The solution is water clear, and gives good results with Feulgen technique.

Leuco-Fuchsine (Fuchsine Sulphurous Acid)

Basic fuchsine	0·5 gm.
Sodium bisulphite	9·0 gm.
Water	500 c.c.

Dissolve the above and add 10 c.c. of hydrochloric acid. Keep in stoppered bottle away from light. The residue of unreduced stain may be removed by filtration through animal charcoal. This gives a truly colourless solution.

Feulgen Stain for Chromatin

Sections are brought into water and then hydrolyzed with *N.*/1 hydrochloric acid. The period varies with the fixative employed.

After a chromic acid fixative hydrolyze at 60° C. for 5–30 minutes. Between these limits the time makes little difference. After other fixatives (for example, formalin-acetic or acetic-sublimate) hydrolyze at 60° C. for 4–8 minutes. Wash.

Stain overnight in leuco-basic fuchsine.

Bring up through graded alcohols to 80 per cent alcohol saturated with sodium carbonate. Leave for 1 hour. Wash in 80 per cent alcohol and dehydrate. Counterstain with fast green or light green, in clove oil or ' Cellosolve '. Wash with xylol and mount.

Congo Red for Nuclei

Congo red - - - - -	1 gm.
Water - - - - - -	100 c.c.

Stain for a few minutes and wash in dilute alcohol. Material should have been fixed in alcohol or acetic alcohol. Ammonium hydroxide 1 per cent may be used as a mordant. Slides should be mounted in xylol-dammar. Balsam is not so good.

Eosine Stain

Eosine - - - - - -	1 gm.
Water or alcohol 70 per cent - -	100 c.c.

A good stain for cytoplasm. May be used in combination with hæmatoxylin or methyl blue.

Various forms of eosine are now on the market, some soluble in water, others in alcohol.

Erythrosine Stain

Erythrosine - - - - -	1 gm.
Water or alcohol 70 per cent - -	100 c.c.

This stain may also be made up in clove oil. It stains rapidly, and 5 minutes is generally sufficient. It should be used last when in combination with other stains, and may replace eosine in combined staining.

Erythrosine Glycerine Stain

Saturated aqueous solution of erythrosine	50 c.c.
Glycerine	50 c.c.

The material is left in this stain for a few minutes, and washed in 50 per cent glycerine. Useful as a "direct" stain or for temporary mounts.

Magdala Red Stain

Magdala red	1 gm.
Alcohol 85 per cent	100 c.c.

The stain is particularly useful for algæ, and may be followed by aniline blue. Stain for 24 hours. It stains lignified and suberized tissues, as well as nuclear material and pyrenoids. It readily fades in sunlight, which may be used to reduce over-staining.

Gentian Violet Stain (Ehrlich) (1)

Gentian violet	1 gm
Alcohol 95 per cent	15 c.c.
Water	80 c.c.
Aniline oil	3 c.c.

A 1 per cent solution in water without aniline keeps better. Gentian violet may also be made up as a 1 per cent solution in clove oil, or equal parts of absolute alcohol and clove oil.

This is a very valuable stain, both for anatomical and cytological work. Gentian violet stains lignified tissue and chromosomes deeply. Flagella and starch are also well stained.

1 per cent aqueous potassium permanganate may be used for 5 minutes as a mordant before the stain. Stain for as long as possible, wash, and dehydrate rapidly.

Crystal violet may be substituted for gentian violet in any stain solution.

Ehrlich's Aniline Water Gentian Violet Stain (2)

Saturated alcoholic gentian violet -	5 c.c.
Aniline water - - - -	100 c.c.

This should be freshly prepared, as it does not keep well.

Aniline Water

Shake up thoroughly 5 c.c. of aniline oil with 100 c.c. water. Allow to stand for a short time and filter through paper.

Gentian Violet-Iodine (Newton)

Fix in Flemming's solution 2–6 hours.

Wash 1 hour.

Grade up through 10, 20, 30, 50 per cent alcohol. Bleach for a few minutes in hydrogen peroxide in 60 per cent alcohol (100 c.c. of 70 per cent alcohol to 10 c.c. hydrogen peroxide). Bring back to water and stain in aqueous 1 per cent gentian violet, 5–7 minutes. Bring up to a solution of 1 per cent iodine and 2 per cent potassium iodide in 70 per cent alcohol. Wash in 85 per cent alcohol, and then in absolute alcohol 3–5 minutes. Differentiate in clove oil.

Newton's Crystal Violet Iodine Stain (La Cour Modification)

Crystal violet 1 per cent in water. Boil and filter. Stain for 10 minutes. (Carnoy material, 3 hr.)

Mordant	Alcohol 80 per cent - -	10 c.c.
	Iodine - - - - -	1 gm.
	Potassium iodide - - -	1 gm.

Treat with mordant for 2 minutes.

Rinse in absolute alcohol. Dip into 1 per cent chromic acid.

Rinse in absolute alcohol. Dip into 1 per cent chromic acid again.

Dip again into absolute alcohol. Clear in clove oil and mount in balsam.

Newton's Crystal Violet Iodine Stain (Smith's Modification)

Mordant in iodine solution. Stain 5–20 minutes in crystal violet and rinse in water.

Dip into iodine solution again, and then into 95 per cent alcohol. Flood the slide quickly with saturated picric acid in absolute alcohol, followed by immediate washing with absolute alcohol for a few seconds.

Differentiate in clove oil and transfer to xylol for 10 minutes before mounting.

Gentian Violet Staining for Pollen Smears (La Cour)

Stain in 1 per cent aqueous gentian violet for 10 minutes. Rinse in water. Dip in absolute alcohol. Dip in alcoholic potassium iodide (1 part iodine, 1 part potassium iodide in 80 per cent alcohol). Dip in absolute alcohol. Chromic acid 1 per cent 15 seconds. Absolute alcohol 5 seconds. Chromic acid 1 per cent 15 seconds. Absolute alcohol 10–15 seconds. Clove oil, xylol. Mount.

Cyanine Stain

Cyanine	1 gm.
Alcohol 95 per cent	100 c.c.
Water	100 c.c.

Dissolve the cyanine in the alcohol and then add the water. Cyanine will not dissolve in 50 per cent alcohol. Stain for 5–10 minutes. This stains lignified tissue blue, and may be used in combination with erythrosine. It also stains chromatin.

Methyl Green Stain (Iodine Green)

Methyl green	1 gm.
Alcohol 70 per cent	100 c.c.

Stain for 24 hours if possible. Only the lignified walls are stained if properly washed. May be used in combination with acid fuchsine, eosine or erythrosine.

Methyl Green Stain (Direct Stain for Chromatin)

Methyl green	1 gm.
Glacial acetic acid	1 c.c.
Water	100 c.c.

For balsam preparations a drop of methyl green and acetic acid should be added to the absolute alcohol when dehydrating. This stain may be used for living tissues or woody sections. When used on living material the acetic acid acts as a fixative, and a pure, direct chromatin stain is obtained.

Light Green Stain

Light green	1 gm.
Clove oil	100 c.c.

or

Light green	1 gm.
Clove oil	75 c.c.
Absolute alcohol	25 c.c.

Stain for about one minute. May be used with safranine, when the clove oil solution will be found most convenient. The stain acts most readily on non-lignified tissues and on spindle fibres. The second method gives a more strongly staining solution.

Malachite Green Stain

Malachite green	2 gm.
Water	100 c.c.

May be used as a stain for cytoplasm in combination with Congo red. A very fast green stain.

Aniline Blue Stain

Aniline blue	1 gm.
Alcohol 85 per cent	100 c.c.

This stains cellulose walls and may be used in combination with safranine. It is also useful for algæ and for staining flagella.

Picro-Aniline Blue Stain

Aniline blue - - - - -	1 gm.
Picric acid 1 per cent aq. sol. -	100 c.c.
Absolute alcohol - - - -	300 c.c.

This stain will be found to be good for algæ. If too strong, it may be diluted with alcohol.

Safranine-Picro-Aniline Blue Stain

Safranine—equal parts of saturated alcoholic and aqueous solutions mixed.

Picro-blue—Picric acid (sat. sol. in alcohol 95 per cent).
Aniline blue (sat. sol. in alcohol 95 per cent).

Mix in proportion of 78 per cent picric acid, 22 per cent aniline blue. Bring sections into 50 per cent alcohol. Stain in safranine for 2 hours, wash in 50 per cent alcohol till light pink. Stain for 2 minutes in picro-blue. Wash in absolute alcohol and dehydrate as rapidly as possible.

Orange *G* Stain

Orange *G* - - - - -	1 gm.
Water - - - - - -	100 c.c.
or	
Orange *G* - - - - -	1 gm.
Clove oil - - - - -	100 c.c.

The clove oil solution may be diluted. This is a first-class plasma stain, and may also be used as a counterstain for sections with lignified tissue which has been previously stained red, blue or green. It is one component of Flemming's triple stain. (See p. 38.) Stain for about half a minute.

Aniline Blue and Orange *G* (Mallory)

Acid fuchsine 1 per cent for 30 seconds.
Distilled water—to 2 minutes.

Stain in:	Aniline blue - - -	0·5 gm.
	Orange *G* - - -	2·0 gm.
	Phosphomolybdic acid -	2·0 gm.
	Distilled water - -	100 c.c.

Stain for 1–5 minutes. Transfer to 1 per cent phosphomolybdic acid for 30 seconds, then to distilled water for 1–2 minutes. Dehydrate and mount.

Gold Orange Stain

Gold orange	1 gm.
Clove oil	100 c.c.

This stain is similar to orange *G*, but is much more soluble in clove oil, and therefore stains much more rapidly.

Bismarck Brown Stain (Vesuvine)

Bismarck brown	2 gm.
Alcohol 70 per cent	100 c.c.

Stain for about 10 minutes. It may be used in combination with gentian violet as a non-lignified tissue stain.

Nigrosine Stain

Nigrosine	1 gm.
Water	100 c.c.

This stain is sometimes used for staining algæ, but is now generally replaced by picro-nigrosine.

Picro-Nigrosine

To 100 c.c. of aqueous saturated picric acid add 1 gm. of nigrosine and filter. This stain keeps well, but fades after about a year. It is excellent for algæ and good for nuclei.

Chlorazol Black *E*

Chlorazol black *E* (sat. sol. in 70 per cent alcohol).

Stain sections for 15–20 minutes.

Nuclei and chromosomes stain black, cytoplasm and inclusions grey, chitin green and glycogen red. May be differentiated with terpineol if overstained.

Polychrome Blue

Polychrome blue	0·5 gm.
Phenol	1·0 gm.
Alcohol 95 per cent	10 c.c.
Water	100 c.c.

Dissolve the phenol in the alcohol and grind up with the stain in an agate mortar. Add two-thirds of the water and stir actively. Pour into a bottle, and wash the mortar with the rest of the water. Stand for 24 hours and filter.

Put a drop of the stain on the section and add a drop of water.

Polychromatic Stain for Fresh Plant Tissues

Thionine	1 gm.
Phenol	2·5 gm.
Water	100 c.c.

Stain for 30 seconds to 2 minutes. Wash in water and mount in either water or glycerine.

Lignified tissues bright blue, pith reddish-purple, phloem and other tissues purple to red. The stain cannot be used for permanent preparations.

Carbol Thionine (Nicolle)

Phenol 2 per cent in water	5 parts
Thionine saturated in alcohol 20 per cent	1 part

Fuchsine and Iodine Green Mixed Solution Stain (1)

Solution *A*	Acid fuchsine	0·1 gm
	Distilled water	50·0 c.c.
Solution *B*	Iodine green	0·1 gm
	Distilled water	50 c.c.
Solution *C*	Absolute alcohol	100·0 c.c.
	Glacial acetic acid	1·0 c.c.
	Iodine	0·1 gm

Mix equal quantities of solutions *A* and *B*, and transfer sections from them directly into solution *C*, and from *C* to xylol.

Zimmermann Stain (2)

Iodine green 0·1 per cent in water	9 parts
Acid fuchsine sat. aq.	1 part

Stain for 10 minutes, differentiate in absolute alcohol with 1 per cent acetic acid and 0·1 per cent iodine.

Auerbach's Stain (3)

Solution *A*	Methyl green - -	1 gm.
	Water - - - -	1,000 c.c.
Solution *B*	Acid fuchsine - -	1 gm.
	Water - - - -	1,000 c.c.

Mix 300 c.c. of solution *A* with 200 c.c. of solution *B*. Stain for 5–15 minutes. Wash in absolute alcohol and destain for up to 1 hour. Heat increases the absorption of methyl green.

Flemming's Triple Stain

Stain in 1 per cent safranine in 50 per cent alcohol for 18–24 hours. Wash off excess of safranine quickly with 50 per cent alcohol. Stain in 1 per cent aqueous gentian violet for 2 hours, rinse in 10 per cent alcohol with a trace of hydrochloric acid. Stain in 1 per cent orange *G* in 50 per cent alcohol for 1 minute. Run up quickly through alcohol and differentiate in clove oil.

Substitution Staining with Free Dye Acids and Dye Bases (McLean)

Make 1 per cent solution of eosine or erythrosine. For every gram of erythrosine add 2·5 c.c. 10 per cent solution of hydrochloric acid of specific gravity 1·16. For every gram of eosine add 3·5 of the same acid. Collect the precipitate formed, and dissolve in xylol at the rate of 200 c.c. per gram of stain used. The solution is colourless. This solution readily stains cellulose walls pink, and may be used as a counterstain at the end of a staining process, immediately before mounting in balsam.

The free base of methylene blue may also be made by adding 2·5 c.c. 10 per cent aqueous solution of sodium hydroxide per gram of dye. Precipitation takes several hours, after which the precipitate may be dissolved in xylol. The xylol solution stains lignified walls blue.

It is not possible to mix the two solutions, but they may be used successively.

Stain for Flagella on Fungal Zoospores (Ledingham)

Place the zoospores on a slide with a few drops of water, invert over a bottle of 1–2 per cent of osmic acid. Leave for a few

minutes for the acid to kill and fix the spores. Stain with the following mixture:

Basic magenta 1 per cent solution -	0·5 c.c.
Crystal violet 1 per cent solution -	1·0 c.c.
Water - - - - - -	25·0 c.c.

Leave the stain on, and dry in a dessicator for 2 hours. Clear the stain with clove oil. Wash in xylol and mount in euparal.

Flagella are stained deep violet; the rest of the zoospore red; the nucleus violet.

Cotton Blue Stain

Saturated solution of cotton blue in alcohol 95 per cent - - -	10 c.c.
Glycerine - - - - -	10 c.c.
Water - - - - - -	80 c.c.

The material is left in the stain for 3 days, during which the spirit and some of the water evaporate. It is then washed in dilute glycerine.

Cotton Blue in Lacto-Phenol

Mix equal quantities of saturated aqueous cotton blue and lacto-phenol. (See p. 17.)

This stain is used for temporary preparations of algæ and fungi. The stain should be gently warmed on the slide and allowed to act for about 30 seconds. Wash in lacto-phenol and mount.

Lacto-Phenol Preparations (Maneval)

Amann's lacto-phenol mounting fluid (see p. 17) may be combined with several stains. Soluble blue and acid fuchsine are good, either separately or mixed. Staining is slow.

Acid Alcohol

Alcohol 70 per cent - - -	100 c.c.
Hydrochloric acid - - -	a few drops

This may be used to destain sections after aniline dyes and it brightens the stain. When used after hæmatoxylin stains, the sections must be afterwards treated with alkaline tap water or

dilute ammonium hydroxide to ' blue ' them. In any event, the sections must be well washed in 70 per cent alcohol after using acid alcohol, or the stains will fade.

BACTERIAL STAINS

Lugol's Iodine Solution (For Gram staining)

Iodine	1 gm.
Potassium iodide	2 gm.
Water	300 c.c.

Gram Gentian Violet Stain

Gentian violet	1·0 gm.
Absolute alcohol	20·0 c.c.
Phenol	2·0 gm.
Water	200·0 c.c.

Counterstain with dilute carbol fuchsine (1 : 9).

Gram's Stain (Jensen's modification)

Stain	Crystal violet 1 per cent in water.
Mordant	Potassium iodide - 2 gm.
	Water - 25 c.c.
	Iodine - 1 gm.
	Dissolve and make up to 100 c.c.
Counterstain	Neutral red 1 per cent in water, for 1–2 minutes.
or	Bismarck brown 1 per cent in water. Boil. Filter.

Dilute 1 : 1 and stain for 1 minute.

Pour on the iodine solution after the crystal violet without intermediate washing. Follow up with a second lot of clean iodine solution. Blot dry. Decolourize with spirit as usual.

Ziehl-Neelsen Carbol Fuchsine

Basic fuchsine	0·3 gm.
Absolute alcohol	10·0 c.c.
Phenol	10·0 c.c.
Water	200·0 c.c.

Dissolve the fuchsine in the alcohol, and then add the phenol dissolved in the water. Apply steaming hot for 10 minutes. Decolourize in 20 per cent sulphuric acid. Counterstain with 1 per cent methylene blue for 2–3 minutes. For the tubercle bacillus.

Claudius Stain for Bacteria

Stain film in 1 per cent aqueous methyl violet 6*B* for 1 minute. Apply half-saturated aqueous picric acid for 1 minute. Remove excess with blotting paper. Decolourize with chloroform or clove oil until no more stain comes out. Apply xylol. Dry.

Stain for Spores (Hewlett)

Flood the slide with carbol fuchsine and warm for 20 minutes. Wash in water for a few minutes, and treat with 1 per cent sulphuric acid for a few seconds. Wash again. Counterstain with methylene blue 1 per cent for 2–3 minutes.

Spore Stain for Bacteria

Lugol's iodine solution - - -	100 c.c.
Eosine, yellowish - - - -	5 gm.
Phenol, crystals - - - -	5 gm.

Filter through paper. Flood fixed film of bacteria with the stain and heat for 5 minutes, bringing it to the boiling point 2–3 times. Wash quickly in distilled water. Counterstain 1½–2 minutes in saturated alcoholic methylene blue, diluted 1 : 20 with distilled water, or with 1 per cent aqueous methylene blue. Rinse, dry and mount. Vegetative cells blue, spores pink.

Zettnow's Flagellar Stain for Bacteria (1)

Cultures must be thoroughly freed from the proteins of the medium by several centrifugings in water or saline.

Mix one drop of 2 per cent osmic acid with one drop of the bacterial emulsion. Make films on slides and air-dry.

Mordant	Tannic acid 5 per cent -	100 c.c.
	Saturated lead acetate - -	1 c.c.

Boil 5 minutes and filter while hot.

Flood films with mordant, heat for a few seconds, wash, and then flood with :

Silver nitrate 3 per cent - - - 40 c.c.
Ammonia, a few drops to render solution opalescent.

Film changes from yellow, by stages, to black. Brief heating may be needed. Wash, dry, mount. Cedar oil causes fading.

Casares-Gil Flagellar Stain (2)

Alcohol 70 per cent - - - - 60 c.c.
Tannin - - - - 20 gm.
Aluminium chloride - - - 36 gm.

Dissolve and add : zinc chloride 20 gm. in 20 c.c. of distilled water. Shake until the precipitate dissolves, after which add 3 gm. of basic fuchsine.

Leifson's Flagellar Stain (3)

Slides must be very clean and strongly flamed before use. Make a dilute suspension of the organisms in water. Place a drop with a wire loop at one end of a slide, and allow the drop to run down the slide. Air-dry.

Flood the unfixed film at about 20° C. for 10 minutes with the following mixture :

Potassium alum, saturated aq. sol. - 20 c.c.
Tannic acid 20 per cent - - - 10 c.c.
Distilled water - - - - 10 c.c.
Alcohol 95 per cent - - - - 15 c.c.
Basic fuchsine sat. in absolute alcohol 3 c.c.

Mix in order given. The mixture may need filtering. It is best after two days, and keeps for one week.

Wash off with water. If a counterstain is desirable for the bodies, stain for 5–10 minutes in the following :

Methylene blue - - - 0·1 per cent
Borax - - - - - 1·0 per cent

Ryo's Flagellar Stain for Bacteria

Combined stain and mordant.

Solution *A*	Phenol 5 per cent - -	10 c.c.
	Tannin - - - - -	2 gm.
	Potassium alum (sat. sol.) -	10 c.c.
Solution *B*	Crystal violet, sat. solution in alcohol.	
	Solutions keep well separately.	

Mix solution *A* 10 parts and solution *B* 1 part at the moment of staining. Stain dried and fixed smears for 3–5 minutes at room temperature. Wash off stain. Dry. Mount in cedar oil.

Loeffler's Bacteria Flagellar Mordant

Tannic acid 20 per cent	10 c.c.
Ferrous sulphate sat. aq.	5 c.c.
Basic fuchsine sat. aq. -	1 c.c.

Loeffler's Flagellar Stain for Bacteria

Aniline water - -	100 c.c.
Sodium carbonate 1 per cent	1 c.c.
Basic fuchsine to saturation.	

Staining Bacteria in Plant Tissues (Wright and Skoric)

Sections should be cut thin, for example, 4μ–6μ.

Bring sections down to water through graded alcohols.

Steep sections for about 1 hour in a buffer solution of about *p*H 6·0.

Stain in Giemsa stain for 10 minutes.

The stain should be freshly made up from the dry powder in the purest, neutral methyl alcohol.

Wash in absolute alcohol until only the bacteria retain the stain.

Dip slides 3–6 times in a 2 per cent solution of safranine in absolute alcohol. Wash in absolute alcohol. Xylol. Balsam.

Bacteria stain dark blue, cell walls red, cytoplasm light blue, and nuclei pink.

STAINS FOR SPECIAL PURPOSES

Stain for Chromosomes in Pollen Tubes

Boil 0·5 gm. agar with 1 gm. sucrose in 25 c.c. of water. Stir in 0·5 gm. powdered gelatine. Smear on warm slide and dust pollen on the film. Germinate in moist chamber. Kill with any good fixative. Stain in 1 per cent potassium permanganate for 3 minutes. Rinse in 5 per cent oxalic acid for 1–3 minutes. Mordant in 1 per cent chromic acid for 20 minutes. Wash, then stain in 1 per cent aqueous crystal violet for 4 hours. Treat with iodine in potassium iodide (1 : 1 : 100 in 80 per cent alcohol). Rinse in 95 per cent alcohol. Counterstain in 1 per cent gold orange in clove oil for 2–4 minutes. Rinse in absolute alcohol, clear and mount.

Chromosomes are intensely stained.

Internal Structure of Chromosomes

Fix in a mixture of 4 per cent phosphoric acid in 4 per cent formalin. Follow by iodine crystal violet stain. (See p. 32.)

Material may be steeped for several hours in 1 per cent uranyl nitrate or acetate as a useful pre-treatment before fixing.

Stain for differentiating Fungal and Host Tissues

Malachite green - - -	0·5 gm.
Acid fuchsine - - - -	0·1 gm.
Martius yellow - - - -	0·01 gm.
Alcohol 95 per cent - - -	50 c.c.
Water - - - - -	150 c.c.

The material should be stained for 15–45 minutes. Remove the excess of stain with 95 per cent alcohol containing a few drops of hydrochloric acid. Mount in balsam after clearing in xylol or carbol turpentine.

Staining Mycelium in Tissues

The sections are placed in alcohol for 10–20 minutes. Then stained in a solution of 0·01 gm. ruthenium red in 15 c.c. distilled

water for 1–3 minutes. In obstinate cases the staining may be prolonged for 12–24 hours and stain heated to boiling point.

Differentiate in 10–20 per cent potassium hydroxide.

Mycelium reddish brown, tissues unstained.

Staining Fungi in Fine Roots (Ivimey Cook)

Mordant the material in 2 per cent iron alum for 12 hours. Wash and place in Heidenhain's hæmatoxylin for 24 hours. Place each root on a clean slide, and wash in water. Drain off water and replace with acid alcohol—4 parts of hydrochloric acid in 96 parts of 95 per cent alcohol. Gently roll out the roots with a glass rod, as the acid alcohol acts. Continue until most of the colour is removed from all but the root tip. Wash in alkaline 95 per cent alcohol. Roots may be counterstained with erythrosine or orange *G*. The fungi, and especially the nuclei, will be stained blue, the cell contents pink or orange.

Stain for Lichens

Stain in 1 per cent aqueous azoblue.

Counterstain with erythrosine in clove oil.

Azoblue has special affinity for the lichen hyphæ.

Stain for Rust Fungi

Fix the material in Gilson's mercuric chloride (see p. 10). Stain with Delafield's hæmatoxylin, followed by eosine.

Stain for Yeast (Wager)

Fix in saturated aqueous mercuric chloride for 12 hours. Wash in water, transfer to 30 per cent alcohol and then to 70 per cent alcohol, and finally to methyl alcohol. Place a few drops of the liquid containing the yeast on a slide and allow to dry. Add one drop of water and allow the slide to dry again. Place the slide in water for a few minutes and stain for 3–10 minutes with a mixture of acid fuchsine and methylene blue or methyl green (see p. 38). Dehydrate rapidly. Differentiate in clove oil, wash in xylol and mount in balsam or glycerine.

Simple Method for staining Yeast Cells (Kelly and Shoemaker)

On a slide place a drop of Mayer's albumen fixative, diluted 1 : 10. Stir into this a small drop of the yeast culture and dry on a warm radiator.

Stain with acid fuchsine 1 per cent for 50 seconds. Wash. Dry and mount.

Simple Stain for Yeast Nuclei (Sander)

Flood film on slide with a saturated aqueous solution of potassium permanganate and heat gently for 2 minutes.

Wash in water. Yeast nuclei are stained deep brown. The same stain may also be applied to bacteria.

Stain for Yeast Spores (Mœller's Method)

Treat the slide with absolute alcohol for 2 minutes, then with chloroform for 2 minutes. Wash in water and treat with 5 per cent chromic acid for 2 minutes. Stain in carbol fuchsine for 10 minutes. Wash. Decolorize in 1 per cent sulphuric acid and wash again. Counterstain in methylene blue 1 per cent for 1 minute.

Staining Pollen Tubes

Small pollinated styles, or longitudinal sections of larger ones, stain well in lactophenol-cotton blue. The stain should be dilute, not more than 0·1 per cent, so that staining may be direct and selective. Material is mounted directly in the staining mixture.

Stain for Protoplasmic Connexions (1)

Make a fresh solution of Hofmann's blue (=soluble blue) in 50 per cent sulphuric acid in a watch glass. Immerse the sections in this for 2 minutes. The protoplasmic connexions are stained blue.

Stain for Protoplasmic Connexions (2)

Aniline blue 1 per cent in 20 per cent alcohol.

Stains much better than gentian violet.

Stain for Protoplasmic Connexions (Meyer) (3)

After fixation and swelling in sulphuric acid (see p. 12) stain in a saturated alcoholic solution of methyl violet, diluted 1 : 30 with 25 per cent sulphuric acid, with the addition of a few drops of iodine solution in potassium iodide as a mordant.

Stain for Cyanophyceæ (McLean)

Methylene blue - - - -	0·1 gm.
Water - - - - -	500 c.c.
Bismarck brown - - -	0·1 gm.
Water - - - - -	500 c.c.

Use equal quantities. These solutions are best made by taking 1 c.c. of 1 per cent Bismarck brown and adding 50 c.c. of water, and 1 c.c. of 1 per cent methylene blue in water and adding 50 c.c. of water. Place a few c.c. in a test tube and drop in the fresh material. Leave for a few days. Soak for 3 minutes in 5 per cent sulphuric acid. Mount in potassium iodide solution saturated with mercuric iodide (see p. 19). For permanent preparations bring up through alcohol and cedar oil and mount in balsam.

Living and Dead Cells (Ruczicka-Tronchet)

Dissolve neutral red and the purest grade of methylene blue in equal quantities, 0·01–0·02 per cent in tap water. Cells are laid in the solution and colour slowly. Blue, if dead, and colourless except for reddish vacuoles, if healthy.

Living and Dead Bacteria

Stain the mixture with methylene blue and then with carbol fuchsine diluted to 1 : 10. Living cells stain blue, dead bacteria red.

Stain for Fats

Spread the material on a slide and add drop by drop 1 per cent Nile blue in alcohol. Then add a drop of water and cover the section. The cells are stained blue, oil drops are stained red.

Permanent Preparation to show Fat in Cells

Stain the section with 1 per cent aqueous chrysoidin, and wash for 1 minute in water. Treat with 10 per cent chromic acid for 1 minute, wash, dehydrate and mount in balsam.

Stain for Latex Tubes

Overstain in safranine and differentiate in 2 per cent acetic acid.

Callose stain

Crystalline sodium carbonate - 4 per cent in water
Rosolic acid (corallin) - - 0·5 per cent in above
Callose stains pink.

Giemsa Stain (Eosine-Methylene Azure)

Should be purchased prepared in solution, as its preparation by the amateur is rarely satisfactory. For unicellular organisms make a smear preparation and invert face downwards into a drop of stain, diluted with 1 c.c. of *neutral* distilled water. Stain 20–30 minutes. Wash, dry and mount.

Smear Preparations for staining of Nuclei

(1) **Root tips.** Fix in a mixture of absolute alcohol 3 parts : acetic acid 1 part, for 12 hours. Hydrolyze in concentrated hydrochloric acid 1 part : 95 per cent alcohol 1 part, for 5–10 minutes. Replace in fixative for 5 minutes. Place on slide in a drop of iron aceto-carmine and press out with scalpel. Place a cover-glass on the preparation and crush flat. Heat 4–5 times over flame, but do not boil. For permanent preparations proceed as given under Belling's iron aceto-carmine. (See p. 27.)

(2) **Anthers.** Spread contents over centre of slide with quick strokes of scalpel or piece of glass. Place the slide face downwards in above fixative, taking care *not* to bring it down obliquely. Fix for 15 minutes, then wash and stain in iron aceto-carmine as above.

Sudan *III* or Scharlach *R* for Cuticle staining

Treat sections first with Eau de Javelle for an hour or longer. This destroys the staining power of lignin, but not of cork or cuticle, both of which stain well in these solutions. Wash well in 1·0 per cent hydrochloric acid.

Sudan III 0·1 per cent in a mixture of equal parts of alcohol and glycerine.

Scharlach R 0·1 per cent in 70 per cent alcohol.

Lay sections in a drop of either dye solution for several hours in the cold. Warming gently hastens staining. Staining is direct. Wash in dilute glycerine and mount in glycerine.

Intra-Vitam staining

The following stains are recommended as non-toxic and as affording a stain which is well retained, though sometimes only in certain cell-elements: methylene blue, Bismarck brown, neutral red, neutral violet, Nile blue hydrochloride or sulphate.

Solutions should lie between the concentrations of 1 : 10,000 and 1 : 100,000.

Methylene blue preparations may be fixed in corrosive sublimate and mounted in glycerine.

A double stain may be obtained by using Bismarck brown, which gives a plasmatic stain, and alum hæmatoxylin diluted 1 : 5,000, which gives a clear nuclear stain.

Other stains may be tried for special purposes, and the following are all relatively non-toxic: Congo red, cyanin, eosine, acid fuchsine, iodine green, Janus green, nigrosine, safranine, soluble blue, toluidine blue, trypan red.

Double-staining Microtome Ribbons (Hill)

(*a*) *For sections which tend to slip off the slide.*

The ribbon is floated on Delafield's hæmatoxylin, and the slide warmed until the sections are quite flat. After a few minutes the hæmatoxylin is drained off and replaced by safranine in 50 per cent alcohol. The stain is again drained off after a few minutes and the ribbon is blotted. The remaining liquid is dried

off in the air, and the sections, with the paraffin, are mounted in balsam.

(*b*) *For sections which do not tend to wash off the slide.*

After staining as above, ribbon is treated with xylol to remove the paraffin before mounting in balsam.

The most satisfactory stain combinations for this method are :

(1) Saturated 50 per cent alcoholic safranine followed by light green in clove oil.

(2) Alcoholic safranine followed by methyl green.

Less satisfactory results may be obtained by methyl green followed by Delafield's hæmatoxylin. Gentian violet followed by Bismarck brown gives poor results.

SYNONYMS OF SOME COMMON STAINS

Acetine Blue	=	Spirit Induline
Acid Fuchsine	=	Magenta Acid. No. 692
Acid Green	=	Light Green S.F. No. 670
Acid Magenta	=	Magenta Acid. No. 692
Acid Rubine	=	Magenta Acid. No. 692
Aniline Blue S.S.	=	Spirit Blue. No. 689
Aniline Blue W.S.	=	Soluble Blues
China Blue	=	Soluble Blues
Coralline	=	Aurine W.S. No. 724
Cotton Blue	=	Soluble Blue. No. 706 or 707
Cyanosine	=	Phloxine. No. 778
Diamond Fuchsine	=	Magenta Basic. No. 677
Dianil Red R.	=	Congo Red. No. 370
Ethyl Eosine	=	Eosine Ethyl. No. 770
Fast Acid Green	=	Light Green S.F. No. 670
Fast Green	=	Malachite Green. No. 657
Fat Ponceau	=	Sudan II. No. 73
Fuchsine	=	Magenta Basic. No. 677
Fuchsine Acid	=	Magenta Acid. No. 692
Gold Orange	=	Tropaeolin O.
Hofmann's Blue	=	Soluble Blues
Hofmann's Violet	=	Dahlia. No. 697

Indulin Black W.S.	=	Nigrosine W.S. No. 865
Iodine Violet	=	Dahlia. No. 697
Isamine Blue	=	Soluble Blue. No. 710
Lauth's Violet	=	Thionine. No. 926
Lyons Blue	=	Soluble Blues
Methyl Blue	=	Soluble Blue. No. 706
Methyl Violet 6 B	=	Benzyl Violet. No. 683
Oil Ponceau G.	=	Sudan III. No. 248
Opal Blue	=	Spirit Blue. No. 698
Phenylene Brown	=	Bismarck Brown. No. 331
Ponceau B.	=	Biebrich Scarlet. No. 280
Ponceau de Xylidene	=	Ponceau 2 R. No. 79
Pyrrol Blue	=	Soluble Blue. No. 710
Rubine Acid	=	Magenta Acid. No. 692
Scharlach R.	=	Sudan IV. No. 258
Toluylene Red	=	Neutral Red. No. 825
Uranine	=	Fluoresceine. No. 766
Vesuvine	=	Bismarck Brown. No. 331
Victoria Green	=	Malachite Green. No. 657
Water Blue	=	Soluble Blues

The numbers given are the reference numbers of the dyestuffs in the *Colour Index*, published by the Society of Dyers and Colourists, Bradford, 1924. Supplement, 1928. (Abstracted from catalogue of the Vector Manufacturing Co., Ltd.)

PLANT SCIENCE FORMULÆ

CHAPTER V

MICROSCOPICAL REAGENTS FOR THE PARAFFIN WAX, CELLOIDIN AND OTHER METHODS FOR MICROTOMY

THE following receipts for use in microscopical work embrace special substances and solutions used in microtome work and the making of microtomed preparations by various methods.

So far as paraffin embedding is concerned, waxes melting between 45° C. and 56° C. will be found the most useful. They may be mixed to give a wax of any particular melting-point. Wax melting at 52° C. will usually be found suitable.

Ceresine

The addition of 0·3 per cent or less of petroleum ceresine to the paraffin wax alters the structure of the whole microcrystalline form and improves infiltration and cutting texture.

Paraffin Wax Infiltration (Dowson)

Paraffin wax - - - - -	1 part
Xylol - - - - - -	2 parts
Absolute alcohol - - - -	3 parts

The mixture solidifies at room temperature. Material in absolute alcohol is warmed in an oven for a few hours, and transferred to the mixture, which is kept corked for 24 hours. The cork is removed and the volatile liquids allowed to evaporate. This is complete in 48 hours. The material is now transferred to pure paraffin wax. Thus the change from alcohol to pure paraffin wax is performed in one operation.

n-Butyl Alcohol for Embedding

Transfer from either 30 or 40 per cent ethyl alcohol, through a mixture of increasing percentages of butyl alcohol to the pure substance. Thence to a mixture of wax and butyl alcohol 2 to 1,

and afterwards to pure wax. Infiltrates slowly, but does not harden, and is therefore recommended for all hard or brittle objects.

Methyl Benzoate for Clearing and Embedding

Clears from 95 per cent alcohol and does not make the material brittle. Its refractive index is 1·517, and it clears slowly. For embedding in paraffin, pass through benzol into wax. It may also be used instead of immersion oil for objectives, and has the advantage of evaporating off the lens without wiping.

Dioxane for Embedding

Dioxane mixes with water, alcohol and molten paraffin wax. Wash material either in water or alcohol. Put it into a jar containing some calcium chloride covered by perforated zinc. Then fill with dioxane. The time of dehydration varies from 4 to 24 hours. Transfer to a mixture of equal quantities of dioxane and paraffin wax. Leave for 1 hour in embedding oven and transfer to pure wax.

Dioxane vapours are toxic, and work must be done under well-ventilated conditions.

Ligroin for Embedding

Ligroin, a petroleum fraction which consists chiefly of heptane and octane, may be used with advantage for paraffin wax embedding. It mixes with 90 per cent alcohol, and it is a better solvent of wax than chloroform. The boiling-point is between 120° C. and 130° C., so that it is fairly volatile.

Ligroin is inflammable, but not dangerously so.

Celloidin and Paraffin Embedding for Large and Difficult Sections

Infiltrate with 2 per cent celloidin in a mixture of equal parts of ether and absolute alcohol. Allow to concentrate in an open vessel for several days at laboratory temperature. Remove surplus celloidin, and place object thus infiltrated in 70 per cent alcohol. Then in chloroform for 2 hours. Transfer to 85 per cent alcohol and embed therefrom in paraffin.

Material cuts at 10μ with surprising smoothness.

Treatment of Objects containing Silica or Other Similar Material

Stems or leaves of grasses infected with rust fungi, or sporangia of *Selaginella*, etc., will cut more easily if treated for a few hours in 5 per cent solution of hydrofluoric acid immediately after fixation.

Soap for Embedding Very Delicate Objects (1)

70 c.c. of hot coco-nut oil are saponified with 38·5 c.c. of 28 per cent potassium hydroxide solution in water. The product is a solid soap which should be dried and powdered before use. Material is placed in warm water and soap powder is added until the mass is solid. Set away to dry, and when sufficiently hard attach to the holder of a sliding microtome.

Mount sections on albumenized slides with a little xylol. Dissolve away the soap with water and immerse in 95 per cent alcohol. Dehydration is avoided by this method.

Embedding Soap for Delicate Tissues (2)

To boiling 20 per cent sodium hydroxide solution add castor oil so long as an alkaline reaction remains. Allow to cool and set. Press and dialyze the mass to remove the last traces of free alkali. Make a 5 per cent solution in distilled water at 40° C., and to each 50 c.c. add 5 c.c. absolute alcohol and 5 c.c. pure glycerine. Objects for embedding are brought into glycerine, and then allowed to steep in the soap solution for 2–3 days. Pour into an open dish and allow to evaporate and set hard, with the aid of gentle heating if necessary. The mass is transparent and cuts well.

Mayer's Albumen Fixative for Sections (1)

White of egg	50 c.c.
Glycerine	50 c.c.
Sodium salicylate	1 gm.

Separate the white from the yoke of the egg, dissolve the sodium salicylate in a very little water and add the other ingredients. The whole is well mixed and then filtered.

Gum Fixative for Sections (Land's) (2)

Gum arabic - - - - -	1 gm.
Potassium dichromate - - -	1 gm.
Water - - - - - -	98 c.c.

Dissolve the gum in water and add the potassium dichromate. Or the gum arabic may be dissolved in one half of the liquid, and the potassium dichromate in the other. The solutions are mixed in equal proportions for use.

Fixative for attaching Sections (3)

Dissolve the maximum quantity of dextrin in water, until a jelly is produced. Then add glycerine and mix thoroughly. The object is to get as much dextrin as possible into a given weight of glycerine. Spread thinly on the slide and then float out the sections, as with albumen fixative.

Fixative for Sections (Haupt) (4)

Dissolve 1 gm. gelatine in 100 c.c. of water at 30° C. Add 2 gm. phenol crystals and 15 c.c. glycerine. Stir well and filter.

CHAPTER VI

PREPARATION OF MUSEUM SPECIMENS

MOST school and many university botanical museums suffer greatly from the poor way in which the specimens are preserved and displayed. This can to a large extent be remedied if greater attention is paid to preserving the colour of the plants immediately they are collected. Many objects exude resinous material after they have been preserved for a short while, and jars should not be sealed down until all resinous matter has dissolved out of the specimen. This is particularly true of storage roots and large cones. A month or more may pass before the jars should be sealed finally.

Flowers may retain their colour better if dried rapidly between blotting papers, especially if hot paper is used; in fact, the quicker the drying process the better the colour will be preserved. They may be preserved in both natural colour *and form* if carefully packed in fine, warm, cedar sawdust and dried in an oven at about 45° C. The best temperature must be found by experiment in each case.

In the preservation of leafy parts of Gymnosperms the material should be plunged into boiling water as soon as collected, otherwise the leaves will drop. Cones may usually be preserved dry, and should be dissected and mounted in glass-topped boxes. Alcohol should not be used for preserving Gymnosperm material; 5 per cent formalin is better.

Mosses may be preserved dry in envelopes, but both mosses and liverworts are best preserved in formalin after greening (see below).

Marine algæ are generally difficult to preserve, since their pigments dissolve out in water. Marine specimens should be washed first in sea water, and then floated out in fresh water on to sheets

of paper or card. A strip of muslin should be placed over the specimen, after which it may be placed between drying paper in a press to dry. Museum specimens should be preserved in 2 per cent formalin in sea water. Members of the Charales are damaged by formalin, and should be preserved in 50 per cent alcohol. They cannot be greened with copper acetate, since the acid dissolves out lime and destroys the specimen.

Fungi, if cut in sections, may be dried between blotting papers. Some retain their colour in 5 per cent formalin; but many require special solutions for this purpose. Mycetozoa and woody Fungi may be preserved dry in glass-topped boxes. Large bracket Fungi can be mounted on boards with brass rods as supports.

All specimens should have their names carefully written, typed or printed, and attached to the jar or box in which they are enclosed. A reference number should also be added, and further details about the specimen recorded in a book or card-index kept in the museum for reference. Specimens should be arranged so far as possible on the shelves in their systematic position. A photograph of the plant in its natural surroundings greatly adds to the value of the specimen. So far as possible handling of specimens should be avoided, for the best sealing cements may give way if they are allowed to come into contact with the preserving fluid too frequently. Glass-fronted cases are best, since these enable the specimens to be well displayed and obviate constant dusting.

COLOUR PRESERVATIVES

Preserving the Green Colour in Plants

A saturated solution of copper acetate in glacial acetic acid is made up, and 20 parts of this are added to 80 parts of water. The specimen is put into this solution at nearly boiling-point, and left for 10 minutes. The liquid is cooled, and the specimen washed and preserved in 5 per cent formalin.

This solution only acts on chlorophyll, and is difficult to use if the material is of a waxy consistency.

Preservative for Coloured Fruits (Hessler's Fluid)

Zinc chloride	200 gm.
Formalin	100 c.c.
Glycerine	100 c.c.
Water	4,000 c.c.

Dissolve the zinc chloride first in the water, and then add the formalin and glycerine. If a sediment appears on cooling, decant off the solution.

Solutions for Preserving the Colour of Fungi (1)

(*a*) Fungi the colour of which is wholly or nearly insoluble in water :

Mercuric acetate	1 gm.
Glacial acetic acid	5 c.c.
Water	1,000 c.c.

(*b*) Fungi the colour of which is very soluble in water :

Mercuric acetate	1 gm.
Neutral lead acetate	10 gm.
Glacial acetic acid	10 c.c.
Alcohol 90 per cent	1,000 c.c.

Solution for Preserving the Colour of Fungi (2)

Zinc sulphate	25 gm.
Formalin	10 gm.
Water	1,000 c.c.

The specimens should be fresh, and must be preserved permanently in the solution.

Preservative for Algæ

Alcohol 50 per cent	90 c.c.
Formalin	5 c.c.
Glycerine	2·5 c.c.
Glacial acetic acid	2·5 c.c.
Copper chloride	1·0 gm
Uranium nitrate	1·5 gm

This makes a good mounting fluid, or alternatively lactophenol with 1·0 per cent of copper chloride may be used.

Preservative for Green Algæ

Place the material in a solution of 0·5 or 1·0 per cent copper acetate in 2 per cent formalin for 24 hours. Preserve in 5 per cent formalin.

MOUNTING SPECIMENS

Gelatine for Mounting Museum Specimens

Small museum specimens may be attached to glass by means of warm gelatine or glycerine jelly. This hardens well, especially if the specimen is mounted in 5 per cent formalin. Care should be taken that the gelatine does not flow across the glass, since it becomes opaque in formalin.

Cement for Sticking Specimens on Opal Glass when Preserved in Alcohol

A quarter of a pound of glue is broken up and covered with sufficient water, and allowed to soak for 12 hours. Excess of water is poured off and the whole boiled in a glue-pot for 3–4 hours. Add one dessert-spoonful of turpentine. Stir well, and boil again until a thick white scum is formed. Skim this off. The solution so obtained is a light golden yellow. The consistency may be adjusted by the addition of more turpentine or by boiling. The glass must be absolutely dry when the liquid is applied. It hardens immediately in alcohol. This medium is also used for sealing lids of museum jars, but is not so satisfactory for this purpose as when kept immersed in alcohol.

Solution for Making Leaf Skeletons

Sodium carbonate - -	4 oz. (150 gm.)
Water - - - -	16 oz. (500 c.c.)

Boil the solution and add 2 oz. (70 gm.) calcium hydrate. Cool and filter. Immerse the leaves and boil for 1 hour. Rub the leaves gently to remove surface tissue. Dry the leaves and mount between sheets of glass.

SEALING MUSEUM JARS

Stockholm Tar for Sealing Museum Jars (1)

A mixture prepared by stirring Stockholm tar into red lead until it becomes chocolate in colour is excellent for sealing lids on museum jars. The mixture should not be made too liquid. It hardens rapidly and cannot be re-liquified, hence only the required quantity should be made at one time. It may be mixed with an old knife on a piece of scrap glass.

Cement for Sealing Rectangular Museum Jars Containing Alcohol (2)

Nelson's No. 2 photographic gelatine	15 gm.
Glycerine	4 c.c.

Soak the gelatine in water for 15 minutes, drain and melt in a glue-pot. When liquid add the glycerine. Drill a small hole in the lid large enough to take a small pipette, in order to fill up the jar after sealing. Place the lid in hot water and apply the hot cement to the edge of the jar. The lid is wiped and quickly applied to the jar. After cooling, an additional coat is applied round the edge, to fill in any gaps left. Further coats may be applied if necessary the following day. Spirit may now be introduced, and the hole in the lid finally closed by means of a cover-glass stuck down with a drop of the medium. This mixture deteriorates with frequent heating, and only a small quantity should be made up at a time.

Asphalt Cement for Museum Jars Containing Formalin (3)

Asphalt	4 parts
Gutta-percha	5 parts

Melt the two over a gas burner in an iron ladle. Stir well so as not to burn. Apply while liquid to the edge of the jar with an old knife. The lid should then be treated similarly. Both should then be warmed near a fire before being pressed into contact. Excess can be trimmed off when cold with the knife.

Sealing Museum Jars (Tagg) (4)

Soak 28 gm. of Nelson's amber gelatine in water for several hours. Pour off water and melt gelatine over water bath. Stir in 0·324 gm. of potassium dichromate. Mix with enough plaster of Paris to give a thick consistency, and run thick layer around the edge of the jar. Warm the lid and press it down in place.

Sealing Museum Jars (Temporary) (5)

Soak one ounce of gelatine for several hours. Pour off water and warm gelatine to melt. Shred into it one-quarter ounce of paraffin wax. Melt together with the gelatine and stir. Apply the mixture warm.

CEMENTS

Cement for Glass

Heat milk with tartaric acid until the casein is coagulated. Drain off the liquid, and dissolve curd by warming with sufficient 6 per cent borax solution.

Cement for Glass

Curdle ½ pint milk with acetic acid. Do not add excess. Mix whey with whites of four eggs, shaking well together. Add quicklime through a fine sieve until the whole forms a paste. Dries quickly and resists heat or water.

Cement for Celluloid

Dissolve scrap celluloid in amyl acetate in such a quantity that the mixture has the consistency of cream. Museum jars may be built up out of celluloid strips and sheets by means of this cement.

De Khotinsky Cement

To 30 gm. of heated pine-tar add about 70 gm. of orange shellac. Heat at about 125° C., with frequent stirring, for 2–4 hours. Test by dropping a little into water. When cold it should break cleanly, without bending.

CUTTING DOWN JAM-JARS

Glass jam-jars may replace museum jars if the tops are cut off. This may be done by making a cut with a glass-cutter round the walls of the jar. To get this even, fix the glass-cutter in a metal vice and rotate the jar against it on the bench.

After a cut has been made, apply very gently a small jet of flame from a fine glass tube, along the cut, until a crack appears. Carefully lead this crack round the jar in the cut made by the glass-cutter, and the top can soon be taken off. The surface may be levelled and smoothed by grinding on a sheet of glass, using emery powder or applying the jar to the side of a fine emery wheel.

The bases of jam-jars cut down in this way find many uses in a botanical laboratory. The tops make excellent dialyzers.

CHAPTER VII

CHEMICAL AND MICROCHEMICAL REAGENTS

THE number of microchemical reagents necessary for botanical work will depend upon the degree of attention which is given to this aspect of botany. It may merely consist of a few bench reagents used for testing sections for the presence of certain common tissue components or plant reserves, or it may become so elaborate that a special biochemical laboratory is set aside for the subject.

In addition to the special receipts given in this chapter, various dilutions of common chemicals such as mineral acids, alkalies and solutions of common salts will be needed. Tables for making up acid dilutions will be found on p. 149. No mention has been made of such solutions in this chapter, since it is impossible to limit the number necessary. Many university botanical departments carry almost as large a variety of stocks of chemicals as that met with in a general chemical laboratory. The solutions are generally prepared in the same way, and are of the same strengths as those used by chemists.

The microchemical tests used in conjunction with a microscope generally depend upon colour changes, and are not always visible if the specimen is mounted in the test solution for microscopic examination. It is always better after treating with a reagent to wash the section in water and mount in either water or glycerine. This method also preserves the stage and other parts of the microscope from damage by corrosive agents.

Iodine Solution (See also Lugol's Iodine Solution, p. 40)

Iodine	1 gm.
Potassium iodide	2 gm.
Water	300 c.c.

First dissolve the potassium iodide in the water and then add the iodine. Potassium bromide may be substituted for potassium iodide.

This is the typical stain for starch, which becomes blue-black. Glycogen stains dark brown. If sections, after staining with iodine, are treated with 60 per cent sulphuric acid, cellulose walls are greatly swollen and are stained blue.

Chloroiodide of Zinc

Zinc chloride - - -	20 gm.
Water - - - - -	8·5 c.c.
Dissolve and cool.	
Potassium iodide - - -	1·0 gm.
Iodine - - - - -	0·5 gm.
Water - - - - -	20·0 c.c.

Add drop by drop to the first solution, until a precipitate of iodine forms, which does not disappear on shaking. This takes about 1·5 c.c.

Gives a violet colour to cellulose.

Chloroiodide of Zinc (Novopokrowsky Formula)

Solution *A*	Iodine - - - -	1 gm.
	Potassium iodide - -	1 gm.
	Water - - - -	100 c.c.
Solution *B*	Zinc chloride - - -	2 gm.
	Water - - - -	1 c.c.

Place sections in solution *A* for a few seconds, and then transfer to solution *B* and move until uniformly stained.

α-Naphthol (Molisch's Reagent for Carbohydrates)

α-naphthol 1 per cent in 70 per cent alcohol.

Mix the solution with a solution of a sugar or other soluble carbohydrate, and pour strong sulphuric acid down the tube. A violet ring is formed at the junction of the two liquids.

Millon's Reagent

Mercury - - - - -	30 c.c.
Nitric acid - - - - -	570 c.c.
Water - - - - - -	1,200 c.c.

Dissolve the mercury in the nitric acid (preferably in a fume cupboard), and then add the water. The solution so formed contains a mixture of mercuric and mercurous nitrates, together with excess of nitric acid and a little nitrous acid.

It gives a red colour with proteins.

Biuret Solution

Sodium hydroxide 20–40 per cent	1 c.c.
Copper sulphate 1 per cent - -	1 drop

Add the sodium hydroxide to the test solution and then the copper sulphate.

A violet colour indicates the presence of protein.

Reduced Oxalic Acid (The Glyoxylic Reaction) (Hopkins & Cole)

Solution *A* 500 c.c. of a saturated solution of oxalic acid are treated with 40 gm. of 2 per cent sodium amalgam. When hydrogen ceases to be evolved the solution is filtered, and diluted with twice its volume of distilled water.

Solution *B* 10 gm. magnesium are covered with distilled water in a flask, and 250 c.c. of saturated oxalic acid are added, cooling under a tap. Filter off the insoluble magnesium oxalate, acidify with acetic acid and dilute with a litre of distilled water.

Mix the solutions in equal proportions with the fluid to be tested, and pour an equal quantity of strong sulphuric acid down the tube. A purple ring at the junction of the liquids indicates the presence of protein.

Russow's Reagent

Mix equal quantities of chlor-zinc-iodide and of a solution of iodine in potassium iodide.

This stains callus deep down.

Phloroglucin

Dissolve the phloroglucin in 95 per cent alcohol, and gradually add strong hydrochloric acid until it begins to precipitate. The solution is then ready for use.

Sections treated with it have their lignified walls stained red.

Aniline Chloride

A saturated solution is made in distilled water, filtered and acidified with a few drops of hydrochloric acid. It should give a distinct acid reaction. The solution may also be made in alcohol and then diluted with water.

Stains lignified walls yellow. Aniline sulphate may be used in place of aniline chloride.

Iodine-Phosphoric Acid (Mangin)

Phosphoric acid, conc. - -	25 c.c.
Potassium iodide - - -	0·5 gm.
Iodine - - - - -	a few crystals

Gently warm. Blot sections free of water and lay in solution. Cellulose colours deep violet.

Chloral Hydrate-Iodine

Dissolve 8 parts of chloral hydrate in 5 parts of water and add iodine crystals, which will dissolve slowly and colour the solution. This solution has a strong swelling and clearing action upon tissues.

It may be used as a test for starch, especially in the chloroplast.

Scharlach Red (Sudan IV) (See also p. 49)

Saturated solution of the dye in 70 parts absolute alcohol and 30 parts water by volume (70 per cent alcohol). Warm to dissolve.

Sudan III (See also p. 49)

Saturated solution of the dye in 70 per cent alcohol. Warm to dissolve. Sections should be left for some time in this stain, and after washing should be mounted in dilute glycerine.

Ruthenium Red for Pectic Compounds

Ruthenium red is ruthenium sesquichloride. Use a 1 : 5,000 solution in water, kept in the dark. Sections should be left for 24 hours in acid alcohol (hydrochloric acid 1 : alcohol 3), and then for some hours in dilute ammonia, followed by the stain.

Colours pectin substances and pectic mucilage deep red.

Test for Pyruvic Acid

Sodium nitro-prusside and ammonium hydroxide in the presence of a little acetic acid give an intense blue colour with pyruvic acid.

Pyruvic acid and 2–4 dinitrophenylhydrazine added to a dilute alkali give an intense red colour.

Glutathione Test

To the tissue preparation in water add ammonium sulphate to saturation, followed by a few drops of a solution of sodium nitroprusside and of strong ammonia.

A pinkish-magenta colour develops, which quickly fades.

Fuchsine (Reduced)

Basic fuchsine which has been carefully decolorized by the addition of strong ammonia, drop by drop, is a test reagent for aldehydes. A purple colour is formed.

For this reason it may be used as a delicate test for the presence of lignin, which reacts by virtue of its higher aldehyde components.

Schiff's Reagent for Aldehydes (Reagent also for Lignin and Cuticle)

Basic fuchsine -	0·025 gm.
Water - - -	100·0 c.c.

When dissolved add 20 drops of concentrated sulphuric acid. Titrate with 1 per cent sodium bisulphite until no more colour is lost. With most samples of fuchsine there will be some residual colour, which is yellowish. Cuticle and lignin stain red, directly, due apparently to their aldehyde content.

Test for Lignin (Maule's Reaction)

Treat the material with potassium permanganate, then wash in dilute hydrochloric acid. Treatment with ammonium hydroxide then gives a red colour, which develops in the lignified walls only. Gymnosperm wood (except *Ephedra*) does not give this reaction.

Test for Inulin

Orcin 0·5 per cent in 90 per cent alcohol.

Sections are treated with this and then warmed in strong hydrochloric acid.

An orange-red colour is produced.

Test for Chitin (Zander) (1)

Treat with Lugol's iodine solution for a short time, then add a drop of saturated solution of zinc chloride. Wash off with water.

Chitin takes a violet coloration.

Stain for Chitin (Bethe) (2)

Stain sections for 3–4 minutes in 10 per cent solution of aniline hydrochloride, to which 1 drop of hydrochloric acid per 10 c.c. has been added. Wash in water and place sections in a 10 per cent solution of potassium dichromate.

When sections are mounted in tap water chitin becomes first green, then blue.

Test for Chitin (3)

Zinc chloride 33 per cent solution in water. Add to every 10 c.c. 3–5 drops of a concentrated solution of iodine in potassium iodide. First soak the material in strong potassium hydroxide, then mount in a drop of the above reagent.

Chitin stains brown outside and violet inside. In some structures all is brown.

Reagent for Distinguishing Fats from Fatty Acids

Boil a solution of Nile blue in dilute (about 10 per cent) sulphuric acid. The original dye is a basic oxazine which gives a blue colour with fatty acids. The sulphuric acid produces also a red oxazine which is not basic and dissolves in fats with a red colour, thus distinguishing fats from fatty acids.

Test for Nitrates (1)

Diphenylamine - - - -	0·1 gm.
Sulphuric acid (nitrogen-free) -	10 c.c.

Gives a bright blue colour when added to a test solution in the proportion of about 5 c.c. of reagent to 2 c.c. of solution.

Test for Nitrates in Plant Tissues (2)

Squeeze out a small drop of the plant juice into the cavity of a well slide. Add a drop of 50 per cent sulphuric acid and a small crystal of diphenylamine. A deep blue colour is produced, which may be used semi-quantitatively by comparing the colour with those produced by solutions of potassium nitrate of known strength.

Test for Nitrates in the Soil (3)

Boil a small quantity of soil for half an hour with four times its own weight of water. Filter, boil and filter again if necessary. Dissolve 0·1 gm. diphenylamine in 10 c.c. strong sulphuric acid. To 1 c.c. of soil extract add 4 c.c. of diphenylamine solution.

A blue colour shows the presence of nitrates.

Nitrites in Soil (Griess-Ilosvay Test) (4)

Boil a small quantity of soil for half an hour with four times its weight of water. Filter, boil and filter again if necessary. Take 3 c.c. of extract and mix with 1 c.c. of 0·5 per cent aqueous sulphanilic acid and 1 c.c. glacial acetic acid. Shake and cool. Dis-

solve 0·1 gm. of α-naphthylamine in 100 c.c. water. Add 1 c.c. to test solution.

Colour is raspberry red, if nitrites are present.

Aniline Acetate Test for Pentoses

Mix equal quantities of aniline oil and glacial acetic acid. This gives a pink colour with pentose sugars, for example, arabinose.

Alkannin Reagent for Fats, Resins and Latex

Make a saturated solution in absolute alcohol, and then dilute with 95 per cent alcohol. It is best to use the dried root of *Alkanna* for making solutions. It may be obtained through a pharmacy. This solution is used for staining oils and resins, but since these are frequently soluble in alcohol they may soon become dissolved. After staining, the sections should be washed and mounted in glycerine. The solution of alkannin does not keep well.

Test for Volutin (Molisch)

Stain living cells with a dilute solution of methylene blue.

Methylene blue (purest grade) sat. aq. 1 part
Water - - - - - - 10 parts

Volutin grains are stained reddish blue to dark blue. Mount the stained cells under a cover-glass and draw 1 per cent sulphuric acid under the cover with a piece of filter paper. All cell structures are decolorized except volutin, which remains dark blue.

Corallin solution (Rosolic Acid) Reagent for Callose.

Make a saturated solution in 4 per cent aqueous sodium carbonate, to which a little camphor has been added as a preservative. Best used fresh.

Phenylhydrazine Hydrochloride for Ozazone Tests

Prepare separate solutions of phenylhydrazine hydrochloride and of sodium acetate in ten times their weight of glycerine. Place a drop of each on the slide and mix. Mount a section of the material in this. Cover with a cover-slip, and heat in an oven at 100° C. for from one to several hours.

p-Nitrobenzene-azo-resorcinol Test for Magnesium

Use a 0·5 per cent solution in 1 per cent sodium hydroxide. Make the material to be tested slightly acid with dilute hydrochloric acid. Add one drop of the reagent, and then more alkali to make the whole slightly alkaline.

A sky-blue precipitate is formed in the presence of magnesium.

Reagent for Potassium (Sodium Cobaltinitrite)

Dilute 3·5 c.c. of glacial acetic acid to 25 c.c. and dissolve in it :

Cobalt nitrite - - - - -	6 gm.
Sodium nitrite - - - - -	9 gm.

Dilute up to 35 c.c.

Sections are laid in a drop of this fluid.

If potassium is present there is a golden precipitate of minute crystals.

Steimetz' Fluid (General Histological Reagent)

Chloral hydrate - - - -	45 gm.
Ferric ammonium alum - -	4 gm.
Aniline sulphate - - - -	1 gm.
Iodine - - - - - -	0·4 gm.
Alcohol 90 per cent - - -	40 c.c.
Glycerine - - - - -	30 c.c.
Distilled water - - - -	25 c.c.

Place the chloral hydrate, alum and 10 c.c. of water in a flask and heat until dissolved. Filter through cotton wool. Dissolve the iodine in the alcohol, and dissolve the aniline sulphate in 15 c.c. of cold water. Mix the three solutions when cold. Add the glycerine and 0·1 gm. of Sudan III. Shake from time to time and filter after 24 hours. Store in a brown bottle. Solution keeps well if well stoppered. Place sections directly in the fluid.

The following colours are produced :

Cuticle and periderm -	red
Lignin - - - -	yellow
Cellulose - - -	colourless or slightly yellow
Mucilage - - -	precipitated, white

Oil, resin or latex	red
Starch - - -	violet blue
Aleurone - -	yellow
Inulin - - -	colourless
Tannin - -	milky blue
Alkaloids - -	brown crystalline precipitate
calcium (for example, middle lamella)	precipitates as tufts of needles

Benzidine

Benzidine 1 per cent in 50 per cent alcohol, followed by a few drops of hydrogen peroxide. Blue colour indicates the presence of peroxidase.

Nessler's Reagent

Potassium iodide - - -	62·5 gm.
Water - - - - -	250 c.c.

Separate 5 c.c. of the solution and then add to the remainder a cold saturated solution of mercuric chloride until a faint precipitate is formed. About 500 c.c. is required. The extra c.c. of potassium iodide is then added, and a few more drops of mercuric chloride added again.

Dissolve 150 gm. of potassium hydroxide in 150 c.c. water, and to the cooled solution the mercury solution above is added. The whole is then made up to 1,000 c.c. The solution is allowed to settle, decanted and the clear fluid kept in a black bottle. This solution may be used as a test for traces of ammonia, by which a brown colour is produced.

Reagent for Indol in Bacterial Cultures (Ehrlich)

Solution *A*	*Para*-dimethyl amido benzaldehyde - -	2 gm.
	Alcohol 96 per cent -	190 c.c.
	Hydrochloric acid conc.	40 c.c.
Solution *B*	Potassium persulphate, saturated solution.	

Add equal amounts of *A* and *B* to the culture in 1 per cent peptone water.

Indol gives a cherry-red colour after the lapse of a few minutes.

Reagent for Intracellular Oxidation. Indophenol Oxidase (Nadi)

(1) M./100 Dimethyl paraphenylene diamine - - - - 1·725 gm. per litre
(2) M./100 α-Naphthol - - - 1·44 gm. per litre
(3) 0·25 per cent aqueous sodium carbonate.

Equal parts of each, freshly mixed.

Oxidizes to indophenol blue.

Manoilov's Reaction

It consists in measuring the power of an extract of tissues to reduce a very dilute solution of potassium permanganate. For example, the amount of an extract of a weighed quantity of tissue in water, or 50 per cent alcohol, which is needed to reduce 3 c.c. of 0·002/*N*. potassium permanganate. The most suitable proportions must be determined in each case.

Fehling's Solution (Strasburger's Formula) (1)

Solution *A*	Copper sulphate - -	35 gm.
	Water - - - -	1,000 c.c.
Solution *B*	Potassium sodium tartrate	173 gm.
	Water - - - -	1,000 c.c.
Solution *C*	Sodium hydroxide -	120 gm.
	Water - - - -	1,000 c.c.

Mix one volume of each of the above with two volumes of water.

Fehling's Solution (2)

Solution *A*	Copper sulphate - - -	34·66 gm.
	Water to - - - -	500 c.c.
Solution *B*	Potassium sodium tartrate -	173 gm.
	Sodium hydroxide - -	50 gm.
	Water to - - - -	500 c.c.

Use equal quantities. Specially adapted for quantitative estimations. A drop of methylene blue solution as an internal indicator greatly sharpens the end-point. The dye is reduced (decolorized) immediately after the Fehling solution has been reduced.

Benedict's Reagent

Solution *A*	Sodium citrate - -	200 gm.
	Sodium carbonate -	200 gm.
	Potassium thiocyanate	125 gm.
	Distilled water - -	800 c.c.
Solution *B*	Copper sulphate -	18 gm.
	Distilled water - -	100 c.c.

Mix by pouring solution *B* slowly into solution *A*. Then add 5 c.c. of a 5 per cent solution of potassium ferrocyanide. Make up with distilled water to 1,000 c.c. The solution keeps indefinitely. This solution may be used for the quantitative estimation of sugars.

Barfoed's Reagent

Neutral copper acetate 5 per cent -	200 c.c.
Acetic acid 40 per cent - - -	5 c.c.

Heated with glucose it gives a red precipitate. No reaction with sucrose or maltose.

Ammoniacal Copper Hydroxide (Schweitzer's Reagent)

To a solution of copper sulphate in water add excess of dilute potassium hydroxide. Filter and collect the precipitate. Wash and dissolve in a little strong ammonium hydroxide. It must be freshly prepared for use. A solvent for pure cellulose.

Selewanoff's Reagent

Resorcinol - - - -	0·05 gm.
Hydrochloric acid 50 per cent -	100 c.c.

Test for lævulose and inulin, in the presence of which a red precipitate appears on boiling.

Jeffery's Macerating Fluid (1)

Nitric acid 10 per cent - - -	50 c.c.
Chromic acid 10 per cent - -	50 c.c.

Macerate for 12–24 hours at a temperature of about 35° C. Wash thoroughly to remove the acid.

Mangin's Macerating Process (2)

Place the tissues for a day or two in a mixture of 3 volumes of alcohol and 1 volume of hydrochloric acid. Rinse in water and place in 10 per cent ammonium hydroxide for 15 minutes. The cells may then be easily separated.

Macerating Fluid (Harlow's Medium) (3)

(1) Place material in strong chlorine water for 3 hours.
(2) Wash.
(3) Place in 3 per cent sodium sulphite solution and warm for 15 minutes.
(4) Wash.

Repeat until the material falls apart.

Schulze's Macerating Fluid (4)

Potassium chlorate - - - -	1 gm.
Nitric acid, conc. - - - -	50 c.c.

Material should be boiled in this mixture for a short while. Solution of the middle lamella causes the cells to fall apart. They should then be washed in water and mounted in glycerine.

Macerating Fluid (Ammonium oxalate) (5)

Boil material with 0·5 per cent ammonium oxalate, which converts pectose into a soluble form.

Eau de Javelle

Add 50 gm. of chloride of lime and 100 gm. of potassium carbonate or sodium carbonate to 1 litre of water. Shake thoroughly. Need not be filtered if it is allowed to settle before use.

Cellulose Acetate Solution

Cellulose acetate	12 gm.
Acetone (pure)	100 c.c.

Chlorophyll Solution

The leaves (dried nettle is good) are steeped in hot water and extracted by shaking with alcohol or acetone. The liquid so obtained contains a mixture of the pigments. The alcohol or acetone solution is gently mixed and shaken with equal quantities of ether and of water. The ether extract is run off, shaken with 30 per cent potassium hydroxide in methyl alcohol, and allowed to settle. The lower layer contains chlorophyll, the upper layer the carotinoids. Carotin may be separated from xanthophyll in the ether layer by washing with water, evaporating nearly to dryness, and then taking up the residue with petroleum. An equal quantity of 90 per cent methyl alcohol is added and the mixture shaken. The methyl alcohol layer contains the xanthophyll, the petroleum layer contains the carotin.

Soap Solution for Surface Tension Measurements

Sodium oleate	2·5 per cent
Glycerine	25 per cent

In distilled water.

Indigo Carmine, reduced

Indigo carmine (sodium indigo sulphonate) is dissolved in tap water at about the rate of 0·1 gm. per litre, so that a layer 10 cm. thick shows a deep sky-blue colour. Make up a solution of sodium hydrosulphite of about 10 per cent in water. Add the latter drop by drop to the indigo carmine solution until the colour is just completely discharged. The reagent is very sensitive to free oxygen, which restores the blue colour.

Quantitative Estimation of Ethyl Alcohol in Fermentation

Potassium dichromate, *N.*/1	0·5 c.c.
Sulphuric acid, conc.	0·5 c.c.

To this mixture add the solution containing the alcohol drop by drop until 0·5 c.c. is added.

Place tube containing the above in boiling water for 5 minutes. Dilute the contents of the tube with cold water. Wash out into a porcelain dish. Titrate with *N.*/50 ferrous sulphate until the yellow colour has nearly given place to green. Use the drop reaction with potassium ferricyanide as the end-point indicator, that is, formation of prussian blue with surplus unoxidized ferrous salt.

150 c.c. *N.*/50 ferrous sulphate is equivalent to 0·5 c.c. *N.*/1 potassium dichromate.

1 c.c. *N.*/1 potassium dichromate is equivalent to 0·0115 gm. ethyl alcohol.

CHAPTER VIII

CULTURE AND NUTRIENT SOLUTIONS

THE number of culture solutions which have been devised is very great, and many of these solutions differ very little from one another except in the proportions of the various ingredients. Culture solutions may be employed either for the cultivation of flowering plants, or for the growth of many algæ, fungi and bacteria. In the case of flowering plants the plants may be grown either directly in the dilute culture fluid, or the solution may be watered at definite intervals of time on to the plants growing in sand, gravel or other porous media.

For Thallophyta the solutions may frequently with advantage be made solid by the addition of 2 per cent agar or 10 per cent gelatine, thus providing a substratum on which they may grow. Thus many of the solutions given in this section might equally well appear in the next section under agars.

In making up culture solutions for studying the growth of higher plants, it is frequently desirable to omit one element from each culture so as to compare the results. The following water culture solution may be cited in this connexion. It will be noted that each salt is replaced by an equivalent weight of a corresponding salt to keep the osmotic pressure constant. It is advisable to replace all monovalents with monovalents, and so forth. In addition to nutrient solutions which are used in this way, many others are employed in the cultivation of moulds, yeasts and bacteria in preference to solid media. Other nutrient solutions are specially adapted for promoting the development of reproductive organs in fresh water algæ. In almost all cases it is very important that a large volume of the liquid should be present in comparison with the quantity of living material, and where possible the solution should be kept in a state of slow movement, or it should be

aerated daily so that fresh oxygenated solution shall be brought into contact with the organisms.

Normal Complete Solution (Sachs)

Calcium sulphate	0·25 gm.
Calcium phosphate	0·25 gm.
Magnesium sulphate	0·25 gm.
Sodium chloride	0·08 gm.
Potassium nitrate	0·70 gm.
Ferric chloride	0·005 gm.
Water	1,000 c.c.

(*a*) *Solution minus Calcium*

For calcium sulphate use potassium sulphate 0·20 gm.
For calcium phosphate use sodium phosphate 0·71 gm.

(*b*) *Solution minus Iron*

Omit ferric chloride.

(*c*) *Solution minus Nitrogen*

For potassium nitrate use potassium chloride 0·52 gm.

(*d*) *Solution minus Phosphorus*

For calcium phosphate use calcium nitrate 0·16 gm.

(*e*) *Solution minus Sulphur*

For calcium sulphate use calcium chloride 0·16 gm.
For magnesium sulphate use magnesium chloride 0·21 gm.

(*f*) *Solution minus Magnesium*

For magnesium sulphate use potassium sulphate 0·17 gm.

(*g*) *Solution minus Potassium*

For potassium nitrate use sodium nitrate 0·59 gm.

Shive's Three-Salt Culture Fluid

Mix gram-molecular solutions in the following proportions :

Potassium phosphate (KH_2PO_4)	18 c.c.
Calcium nitrate	5 c.c.
Magnesium sulphate	15 c.c.
Water	1,000 c.c.

Add to each litre 10 drops of a suspension of 0·2 gm. ferric phosphate in 100 c.c. of water.

Pfeffer's Solution

Calcium nitrate	4 gm.
Potassium nitrate	1 gm.
Magnesium sulphate	1 gm.
Potassium phosphate (KH_2PO_4)	1 gm.
Potassium chloride	5 gm.
Ferric chloride	trace
Water	3–7 litres

Sand Culture Solution for Watering (1)

Solution *A*	Magnesium sulphate	50 gm.
	Water	500 c.c.
Solution *B*	Potassium phosphate (KH_2PO_4)	50 gm.
	Water	500 c.c.
Solution *C*	Calcium nitrate	50 gm.
	Calcium chloride	50 gm.
	Water	500 c.c.
Solution *D*	Ferric chloride	4 gm.
	Water	300 c.c.

Add 10 c.c. of *A*, *B* and *C*, with 10 drops of *D*, to 4 litres of water.

Solution for Watering Sand Cultures (2)

Magnesium sulphate	0·6 gm.
Potassium chloride	0·7 gm.
Potassium phosphate (KH_2PO_4)	0·7 gm.
Ammonium nitrate	2·3 gm.
Ferric chloride	trace
Water	10 litres

Where plants are infected with root-nodule bacteria, omit the ammonium nitrate.

Wagner's 'Nährsalz' Mixture for Watering Sand Cultures (3)

Ammonium phosphate	15 gm.
Potassium nitrate	15 gm.
Potassium chloride	5 gm.
Sodium nitrate	25 gm.
Ammonium sulphate	40 gm.

Add 10 gm. of the mixed salts to 10 litres of water. Give $\frac{1}{8}$–1 litre of solution. If plants are growing quickly, water once a week, if slowly once a fortnight. Water between times with pure water. Apply with a rose.

Cohn's Nutrient Solution

Potassium phosphate (KH_2PO_4)	5·0 gm.
Magnesium sulphate	5·0 gm.
Ammonium tartrate	10·0 gm.
Potassium chloride	0·5 gm.
Water	1,000 c.c.

Fermi's Culture Fluid

Magnesium sulphate	0·2 gm.
Potassium phosphate (KH_2PO_4)	1·0 gm.
Ammonium phosphate	10·0 gm.
Glycerine	45·0 c.c.
Water	1,000 c.c.

This may be added to agar or peptone beef broth or silica jelly, in which case the volume of water must be reduced.

Frænkel and Voges' Solution

Sodium chloride	5 gm.
Potassium phosphate (K_2HPO_4)	2 gm.
Ammonium lactate	6 gm.
Sodium asparaginate	4 gm.
Water	1,000 c.c.

Mayer's Culture Fluid

Magnesium sulphate	10·0 gm.
Ammonium nitrate	15·0 gm.

Calcium phosphate ($Ca_3(PO_4)_2$)	0·1 gm.
Potassium phosphate (KH_2PO_4)	10·0 gm.
Water - - - - - -	1,000 c.c.

Dissolve cold and add whatever sugar is necessary as a carbon source. 3 per cent sodium chloride may be used for luminous bacteria or excess of calcium carbonate for acid-forming bacteria.

Molisch's Culture Medium

(Gelatine - - - - -	100 gm. if desired solid)
Sucrose - - - - -	20 gm.
Peptone - - - - -	10 gm.
Potassium phosphate (K_2HPO_4)	0·25 gm.
Magnesium sulphate - -	0·25 gm.
Water - - - - -	1,000 c.c.

Enough sodium hydroxide is added to make the solution fully alkaline. Luminous bacteria grow slowly on this medium, but develop rapidly and become luminous when 3 per cent sodium chloride is added.

Nægeli's Nutrient Solution

Calcium chloride - - -	0·1 gm.
Magnesium sulphate - -	0·2 gm.
Potassium phosphate (K_2HPO_4)	1·0 gm.
Ammonium tartrate - - -	10·0 gm.
Distilled water - - - -	1,000 c.c.

Prazmowski's Culture Fluid

Potassium phosphate (K_2HPO_4)	5 gm.
Magnesium sulphate - -	5 gm.
Ammonium carbonate - -	5 gm.
Calcium chloride - - -	0·5 gm.
Water - - - - -	1,000 c.c.

Dissolve cold. Sugar may be added as a carbon source.

Raulin's Culture Fluid

Cane sugar	70·00 gm.
Tartaric acid	4·00 gm.
Ammonium nitrate	4·00 gm.
Ammonium phosphate	0·60 gm.
Potassium carbonate	0·60 gm.
Magnesium carbonate	0·40 gm.
Ammonium sulphate	0·25 gm.
Zinc sulphate	0·07 gm.
Ferrous sulphate	0·07 gm.
Potassium silicate	0·07 gm.
Water	1,500 c.c.

Uschinsky's Solution

Ammonium lactate	6–7 gm.
Sodium asparaginate	3–4 gm.
Sodium chloride	5–7 gm.
Potassium phosphate (K_2HPO_4)	2–2·5 gm.
Magnesium sulphate	0·2–0·4 gm.
Calcium chloride	0·1 gm.
Glycerine	30–40 gm.
Distilled water	1,000 c.c.

Knop Solution for Algæ

Potassium nitrate	1 gm.
Magnesium sulphate	1 gm.
Calcium nitrate	3 gm.
Potassium phosphate (K_2HPO_4)	1 gm.

Dissolve the first, second and fourth in a litre of water, and then add the calcium nitrate. A white precipitate is formed, and the stock solution must be shaken before use.

This is a 0·6 per cent solution, and is best for inducing the fruiting condition in most Chlorophyceæ.

Benecke's Solution

Calcium nitrate	0·5 gm.
Magnesium sulphate	0·1 gm.

Potassium phosphate (KH_2PO_4)	0·2 gm.
Ferric chloride - - - -	a trace
Water - - - - -	1,000 c.c.

This solution is recommended for the cultivation of algæ.

Tubeuf's Culture Medium for Dry-Rot Fungus

Ammonium nitrate - - -	10 gm
Potassium phosphate (KH_2PO_4) -	5 gm
Magnesium sulphate - - -	1 gm
Lactic acid - - - -	2 gm
Water - - - - -	1,000 c.c.

Lodder's Potato Water for Yeasts

Grated potato pulp - -	20 gm
Water - - - -	1,000 c.c.

Allow to stand for 4 hours. Steam for 15 minutes. Filter, tube and autoclave for 30 minutes at 120° C.

Hansen's Medium for the Culture of Yeasts

Peptone - - - - -	1 gm.
Maltose - - - - -	5·9 gm.
Potassium phosphate (KH_2PO_4) -	0·3 gm.
Magnesium sulphate - - -	0·2 gm.
Water - - - - -	100 c.c.

Another Formula

Peptone - - - - -	1 gm.
Dextrose - - - - -	5 gm.
Potassium phosphate (KH_2PO_4) -	0·3 gm.
Magnesium sulphate - - -	0·5 gm.
Water - - - - -	100 c.c.

Leberle-Will Culture Medium for Yeasts

Calcium phosphate ($CaHPO_4$) -	0·50 gm
Potassium phosphate (K_2HPO_4)	4·55 gm
Magnesium sulphate - - -	2·10 gm
Peptone - - - - -	20·00 gm
Water - - - - -	1,000 c.c.

Pasteur's Culture Fluid for Yeasts (Original formula)

Ammonium tartrate	10 gm.
Ashes of yeast	10 gm.
Rock candy	100 gm.
Distilled water	1,000 c.c.

Dissolve cold.

Modified Pasteur's Solution for Yeast Cultivation

Potassium phosphate (KH_2PO_4)	2·0 gm
Calcium phosphate ($CaHPO_4$)	0·2 gm
Magnesium sulphate	0·2 gm
Ammonium tartrate	10·0 gm
Glucose	150 gm.
Water	2,000 c.c.

Synthetic Medium for Yeasts

Sucrose	100 gm.
Ammonium chloride	1·2 gm
Potassium phosphate (K_2HPO_4)	0·5 gm
Calcium chloride	0·1 gm
Magnesium sulphate	0·2 gm
Distilled water	1,000 c.c.

Yeast Water Medium

Autoclave sterile, fresh, starch-free, baker's yeast in 10 times its weight of tap water. Allow to settle and decant off the clear liquid. Then add to each 100 c.c. :

Mannite	10 gm.
Magnesium sulphate	0·2 gm.
Potassium phosphate (K_2HPO_4)	0·5 gm.
Sodium chloride	0·1 gm.
Distilled water	900 c.c.

Hopped Beerwort for Cultivation of Fungi

Beerwort of specific gravity about 9° Balling (=9 per cent sugar) is sterilized 20 minutes in steam, 3 times, with 24-hour intervals. Heating should be as short as possible to avoid caramelization. Keep at room temperature for 14 days before use, to pick up oxygen and check sterility.

Unhopped Beerwort for the Cultivation of Fungi

Unhopped beerwort of specific gravity 5° Balling (=5 per cent sugar) is made up by diluting, if necessary, wort from the mash tun. Sterilized and treated as for hopped wort.

This may also be made synthetically from concentrated malt extract, diluted to the above strength. Peptone may also be added, 5–10 gm. per litre of wort.

Beerwort for Culture Media

Malt 200 gm. Pound up and soak in 1 litre of cold water. Bring up slowly to 60° C. Keep at this temperature for 45 minutes. Add 4 gm. of hops. Boil 1 hour. Filter. Test for maltose and dilute to give a final concentration of 3 per cent. May be solidified with 2 per cent of agar or 8 per cent of gelatine.

Directions for making Plant Decoctions

Dry chopped substance	50 gm.
Water	1,000 c.c.

Cook over a burner and boil for 15 minutes, stirring to prevent burning. Filter, clear with white of egg if necessary, and make up to 1,000 c.c.

Hay Infusion for Bacillus subtilis

Chopped hay	10 gm.
Water	1,000 c.c.

Heat to 70° C., plug flask with cotton wool and macerate in a water bath for 3 hours at 60° C. Make up the liquid to the original volume and sterilize at 100° C. for 1 hour. Only spores of the *subtilis* type will survive in the liquid after this treatment.

Ringer's Fluid

Sodium chloride	7·5 gm.
Potassium chloride	0·075 gm
Calcium chloride	0·1 gm.
Sodium bicarbonate	0·1 gm.
Distilled water	1,000 c.c.

Dissolve in order given. It is a balanced observation fluid for most living cells.

Artificial Sea Water

Based on the composition of the sea water at Naples.

Sodium chloride	29·42 gm.
Potassium chloride	0·5 gm.
Magnesium chloride	3·22 gm.
Sodium bromide	0·56 gm.
Calcium sulphate	1·36 gm.
Magnesium sulphate	2·40 gm.
Calcium carbonate	0·11 gm.
Ferric oxide	0·003 gm.
Water to	1000 c.c.

For aquaria it is best to add to this quantity a few cubic centimetres of a nutrient water-culture solution to compensate for the lack of movement in the water.

BACTERIOLOGICAL LIQUID MEDIA

Beef Extract Bouillon

Lean meat, minced	500 gm.
Water	1,000 c.c.

Place in a cool room for 12 hours, stirring occasionally, or it may be warmed to 65° C. for 1 hour, though the former method is better. The red liquid is filtered through cheese cloth and made up to 1 litre. Add:

Peptone	10 gm.
Sodium chloride	5 gm.

Autoclave for 15 minutes and decant the supernatant liquid. Neutralize the solution with sodium hydroxide to *p*H 7·4.

Plain Lemco Broth for Bacteria

"Lab" Lemco	4 gm.
Peptone	10 gm.
Sodium chloride	5 gm.
Water	1,000 c.c.

Adjust to *p*H 7·4, filter, and sterilize in the autoclave at 115° C. for 15 minutes.

Litmus Milk

Milk	100 c.c.
Saturated litmus solution	2 c.c.

Skim milk should be used, or if not, the cream must be separated by standing or by centrifuging. Autoclave at 110° C. for 15 minutes. Brom-cresol purple solution, *quant. suff.*, may be substituted for litmus.

Conditions for Anærobic Cultures of Bacteria

An air volume of 400 c.c. is rendered oxygen-free in 30 minutes by a mixture of 10 c.c. of 20 per cent potassium hydroxide with 3 c.c. of 44 per cent pyrogallol. Most absorption takes place in the first half-hour. An oxygen indicator may be made with cotton wool damped with the following solution, which bleaches as oxygen disappears.

Glucose 10 per cent	4·2 c.c.
N. Sodium hydroxide	0·1 c.c.
Methylene blue, sat. sol.	0·1 c.c.

Sodium Pyrogallate for Oxygen Absorption

Solution *A* Dissolve sodium hydroxide in an equal weight of water.

Solution *B* Dissolve pyrogallic acid in three times its weight of water.

Mix 5 parts of *A* with 2 parts of *B*. Gives off a minimal amount of carbon monoxide and absorbs rapidly, at rate of 100 c.c. of oxygen in 8½ minutes per 35 c.c. absorbent.

CHAPTER IX

AGAR AND OTHER NUTRIENT SOLID MEDIA

NUTRIENT agars are used in the botanical laboratory mainly for the cultivation of fungi. The great majority of common fungi may be satisfactorily grown from generation to generation in this way, and are thus available at short notice for class work. In addition to fungi, bacteria and to some extent algæ may also be grown in this way.

The chief requirements of an agar are first that it shall supply a substratum on which the organism may grow, and secondly that it shall provide the necessary food for the organism's metabolism. There is much to be said in favour of synthetic agars for this purpose, since it is possible to modify the quantities of the various ingredients (including vitamins or other accessory substances where needed), so as to provide the most suitable food for the fungus. On the other hand, for ordinary stock cultures the food may be provided more simply by making use of natural plant, or occasionally animal, material, dissolving it in water and solidifying it with agar. Some fungi which will not grow well on synthetic media may be cultivated in this way.

Many of the synthetic nutrient solutions mentioned in the last chapter may be used for this purpose, being solidified by the addition of 2–3 per cent of agar. Less than 2 per cent of agar provides a medium that is not sufficiently solid, while more than 3 per cent is only required in hot countries.

The preparation of media presents certain difficulties. Agar can only be filtered in a hot state, and where possible the solution should be filtered before the addition of the agar. Filter pulp or paper pulp spread in a layer about one half-inch thick on the bottom of a Jena-glass Buchner funnel and moistened with hot water will be found very convenient. If the funnel is fitted in a

filter flask and coupled to a good water-pump, hot agar may be filtered very quickly and cleanly, without egging. Slow filtration through paper with a hot-water funnel is rarely as successful as this method. In any case, decanting the liquid off any undissolved residues before the filtration of agar will always facilitate the process.

Agar may be most conveniently stored in screw-cap bottles of about 200 c.c. capacity, that is, 8-oz. "medicine flats". They may be sterilized in these with the screw-caps slightly loosened. Most agars require 15–20 minutes at a temperature of 115° C. to sterilize them. The autoclave should be allowed to cool almost completely before the bottles are removed, and the caps of the bottles should be immediately screwed up tightly. If kept in a cool place or refrigerator, media prepared in this may be kept for months or even years in perfect condition. When needed, the bottles are taken out, the stoppers slightly unscrewed and the whole placed in a steamer until melted.

Petri dishes should not be autoclaved. They may be heated to 150° C. for half an hour in a hot-air oven, after being wrapped separately in newspaper or white kitchen-paper, in which they are kept until required. 10–15 c.c. of agar in a test tube is melted in a water bath and poured into a plate immediately before it is required. Small screw-capped bottles of 20 c.c. capacity are on the market, which can with advantage be used to replace test tubes for cultures. They are treated in just the same way, but have the advantage that the medium does not dry up so quickly as in tubes plugged with cotton wool. For those fungi which require oxygen the cap must not be tightly screwed up.

Cotton wool of varying colours may be used for plugging tubes, and will be found useful for distinguishing agars of varying composition. Another method is to drop glass beads of various colours into the stock bottles. Labels and names written on glass usually disappear during sterilization, but the "Kum-Klean" adhesive labels are apparently proof against steam.

All media should be used at a known pH, and stocks of media should be standardized by titration, while hot, against standard

acid or alkali, and the requisite amount of acid or alkali added to the bulk of the medium to bring it to the required *p*H. Since bacteria grow best on alkaline media, their growth may be kept down by using more acid media. This is particularly useful in making isolations of fungi from mixed cultures.

For most bacteria media should lie between *p*H 7·2 and 7·4. Fungi prefer media of about *p*H 5·0, though many require still greater acidities. The *p*H must be judged by comparison with a buffer solution of the required reaction, containing the indicator.

The most generally useful temperatures for cultures are 25° C., 28° C. and 37° C. Thermostats must be accurate, so that the temperature does not vary more than 1° C., and care should be taken to see that the temperatures at the top and bottom of the oven are reasonably similar. Electric incubators are the most satisfactory. For keeping stock cultures and media, a refrigerator working at about 4° C. will be found invaluable.

Bacteriological Agar; Determination of *p*H

Nutrient agar is so well buffered that it will stand sevenfold dilution without change of *p*H.

At this dilution it does not set when cold, which greatly facilitates titration.

Beerwort Agar

2 per cent beerwort agar may be made by taking 2 gm. of agar, dissolving it in 100 c.c. of water in a steamer, and adding 2 per cent beerwort to the liquid. Filter through paper pulp before use.

Media for Yeast Sporulation (Gorodkowa)

Agar	10 gm.
Peptone	10 gm.
Lemco extract	10 gm.
Sodium chloride	5 gm.
Dextrose	2·5 gm.
Water	1,000 c.c.

At 28° C. spores are produced in 3–4 days. At room temperature they take much longer to appear.

McKelvey's Medium for Yeast Sporulation

Chopped or grated carrot - -	100 gm.
Water at 60° C. - - - -	1,000 c.c.

Shake well for about 1 hour. Warm up to the original temperature, and keep standing at this temperature for 2–3 hours. Filter through flannel. Add 2 per cent agar. Steam until dissolved. Tube out into test tubes for slants, adding about one-half teaspoonful of calcium sulphate to each tube.

Cellulose Agar

Make up a solution of ammonium hydroxide (spec. grav. 0·90) 10 parts to water 3 parts. Add copper carbonate until precipitate is no longer dissolved, and allow to stand overnight, after shaking well. Add 10 gm. of filter paper or paper pulp and shake until dissolved. Dilute to 10 litres and add slowly a 1 in 5 solution of hydrochloric acid, shaking vigorously until all the cellulose is precipitated. Dilute to 20 litres. Allow the cellulose to settle and decant off the supernatant liquid. Wash the residue with water, adding hydrochloric acid if the blue colour of the copper salt reappears; then with water to remove the chlorine. Allow to settle and decant off the liquid. Centrifuge to deposit the cellulose if necessary. Then make up the agar:

Cellulose suspension - - -	500 c.c.
Agar - - - - - -	15 gm.

To this add the following nutrient solution:

Potassium phosphate (KH_2PO_4) -	1 gm
Magnesium sulphate - - -	1 gm
Sodium chloride - - -	1 gm
Ammonium sulphate - - -	2 gm
Calcium carbonate - - -	2 gm
Water to - - - - -	1,000 c.c.

Corn-Meal Agar (1)

Corn (maize) meal - - -	50 gm
Water - - - - -	1,000 c.c.

Warm at a temperature of 58°–60° C. for 1 hour. Filter and add 1·25 per cent of agar. Steam for 90 minutes. Filter and tube. Autoclave for 15 minutes at 115° C.

Corn Meal Agar (2)

Corn (maize) meal	300 gm.
Water	1,000 c.c.

Boil for 15 minutes, and then decant off the clear liquid. Make up with 20 gm. of agar per litre of clear fluid.

Malt Corn-Meal Agar (Lister Institute)

Mix 50 gm. of crushed maize with 1 litre of water and autoclave for 2 hours at 120° C. Cool to 60° C. and add 1 tablespoonful of malt extract. Keep at the above temperature for 30 minutes. Filter and add 2 per cent of agar and steam for 30 minutes. Tube and sterilize by steaming.

Malt Agar

Malt extract	10 gm.
Agar	7·5 gm.
Water	500 c.c.

Dissolve the agar in the water in a steamer and filter through paper pulp. Add the malt extract dissolved in a little hot water. Autoclave at 115° C. for 20 minutes.

Dung Agar

About 1,000 gm. of horse, cow or rabbit dung are soaked in cold water for three days. The liquid is poured off and diluted with cold water until it is a pale straw colour. 25 gm. of agar are added to every litre of the solution, which is then sterilized in the autoclave.

Potato Agar (1)

Grate 500 gm. of clean, peeled potatoes into 500 c.c. of water. Allow to stand for 6–8 hours, without boiling. Filter off the solution and add 20 gm. of agar in 500 c.c. of water. Steam for ½ hour. Filter through paper pulp and autoclave for 20 minutes at 115° C.

Potato Agar (2)

About 250 gm. of potatoes are peeled, cut into small pieces, covered with water and boiled gently for about half an hour. The extract is allowed to cool and settle as much as possible, and the supernatant liquid is poured off and made up to 1,000 c.c. with water. It is placed in a flask with 25 gm. of agar, steamed until dissolved and then sterilized in the autoclave.

Prune Agar (1)

25 prunes are boiled for an hour, and the liquid poured off and made up to 1,000 c.c. 25 gm. of agar are added, and the whole steamed, filtered through paper pulp and sterilized.

Prune Agar (2)

Boil 4 prunes in 1,000 c.c. water for 1 hour, taking care that the skin is not broken. Strain through muslin and make up the volume with water. Add 2 per cent of agar and steam for ¾ hour. Filter through paper pulp. Autoclave for 15 minutes at 115° C.

Prune Agar (Milburn) (3)

Soak 1 lb. of prunes in 1 litre of water for 12 hours. Filter through cloth. Concentrate to a specific gravity of 1·06–1·08. Use this in the proportion of 40 per cent for making up 2 per cent agar, and add casein as a nitrogen source.

Brown's Synthetic Medium for Fungi

Agar - - - - -	7·5 gm.
Water - - - - -	500 c.c.

Dissolve the agar in the water, and then add the following :

Glucose - - - - -	1 gm.
Asparagine - - - -	1 gm.
Potassium phosphate (K_3PO_4) -	0·625 gm.
Magnesium sulphate - -	0·375 gm.

Autoclave for 15 minutes at 115° C.

Standard Agar for Fungi (Das Gupta Formula)

Magnesium sulphate	0·75 gm.
Potassium phosphate (K_3PO_4)	1·25 gm.
Asparagine	2·00 gm.
Glucose	2·00 gm.
Potato starch	10·00 gm.
Agar	15·00 gm.
Water	1,000·00 c.c.

This medium gives good zonation and colour to cultures, with practically linear growth-rate.

Czapek-Dox Agar

Sucrose	30 gm.
Sodium nitrate	2 gm.
Potassium phosphate (K_2HPO_4)	1 gm.
Magnesium sulphate	0·5 gm.
Potassium chloride	0·5 gm.
Ferrous sulphate	0·01 gm
Agar	15·00 gm
Water	1,000·00 c.c.

For Mucoraceæ substitute glucose for sucrose.

Sabouraud's Agar for Fungi

Maltose	40 gm.
Peptone	10 gm.
Agar	15 gm.
Water	1,000 c.c.

Needs no adjustment. Sterilize at 100° C. only. Crude maltose is best. An equal weight of crude glucose may be substituted in certain cases.

Conn's Agar

Potassium nitrate	2·00 gm.
Magnesium sulphate	1·20 gm.
Potassium phosphate (KH_2PO_4)	2·70 gm.
Maltose	7·20 gm.

Potato starch	10·00 gm.
Agar	15·00 gm.
Water	1,000·00 c.c.

Gives a good spreading effect.

Richard's Agar

Potassium nitrate	10·00 gm.
Magnesium sulphate	0·25 gm.
Potassium phosphate (KH_2PO_4)	5·00 gm.
Sucrose	50·00 gm.
Potato starch	10·00 gm.
Ferric chloride	trace
Agar	20·00 gm.
Water	1,000·00 c.c.

This agar gives a good aerial mycelium, but strains are not easily distinguishable.

Barnes' Agar

Potassium phosphate (K_3PO_4)	0·1 gm.
Ammonium nitrate	0·1 gm.
Potassium nitrate	0·1 gm.
Glucose	0·1 gm.
Agar	2·5 gm.
Distilled water	100·0 c.c.

Clausen's Agar for Ascomycetes

Inner dish

Potassium phosphate (KH_2PO_4)	0·05 gm.
Ammonium nitrate	0·05 gm.
Magnesium sulphate	0·02 gm.
Ferrous phosphate	0·001 gm.
Agar	3·00 gm.
Distilled water	100·00 c.c.
Inulin	2·00 gm.

Outer dish

The same as the above, but without inulin.

Winogradsky's Culture Medium for Nitrogen Fixing Bacteria

A 4 per cent solution of silicic acid in distilled water is placed in flasks. On the addition of the following salts gelatinization occurs.

Solution *A*	Ammonium sulphate -	0·4 gm.
	Magnesium sulphate -	0·05 gm.
	Calcium chloride -	a trace
	Water - - -	50 c.c.
Solution *B*	Potassium phosphate (KH_2PO_4) - -	0·1 gm.
	Sodium carbonate -	0·6–0·9 gm.
	Water - - -	50 c.c.

Mix in equal quantities and sterilize. Then add in small quantities to the silicic acid.

Nutrient Gelatine

Gelatine - -	40 gm.
Peptone - -	5 gm.
Lemco extract	5 gm.
Sodium chloride	2·5 gm.
Water - -	500 c.c.

Glucose-Gelatine (1)

As above, with the addition of 1–2 per cent of glucose. The glucose should be added last and the medium sterilized only at 100° C.

Glucose-Gelatine (2)

Peptone - - -	6 gm.
Sodium chloride -	3 gm.
Beef extract - -	3 gm.
Dextrose - -	6 gm.
Gelatine - - -	60 gm.
Water -	600 c.c.

The gelatine is added when the water containing the other ingredients is brought to the boil. The mixture is cooled to 60° C., and the white of two eggs is added and stirred well. The

liquid is then steamed for 10 minutes and filtered through paper pulp.

Plain Lemco Agar

Add 2 per cent of agar to Lemco broth (see p. 87) to solidify it. Filter. Adjust *p*H. Sterilize at 115° C. for 15 minutes.

Glucose-Agar

As above, with the addition of 2 per cent glucose. Sterilize at 100° C. only.

Medium for Separating Fungi and Bacteria

The addition of 0·2 molar solution of potassium thiocyanate to agar prevents bacterial growth, but does not hinder fungi. The concentration should be such that there is 0·1 gm. of thiocyanate to each 5 c.c. of agar medium.

Moist Chambers for Micro-culture of Fungi

(1) Balsam two small strips of glass, not above 1 mm. thick, to a microscope slide, at $\frac{3}{4}$ in. apart. When dry lay a square $\frac{3}{4}$ in. cover-glass on these, fixing it down with wax or vaseline. Run in a drop of prune agar, charge with spores, at one end of the shallow chamber thus formed, and close the other, open, end with a plug of cotton wool.

(2) *Nemon's.* Place a large drop of agar or culture medium on a slide, and flatten it out under a large cover-glass. When set, lift the cover and cut the flat cake of medium in halves, separating them slightly with the knife. Sow the flat edge of one half with spores and replace the cover-glass.

Egg Albumen Agar for Actinomycetes (Waksman)

Dextrose	10 gm.
Potassium phosphate (K_2HPO_4)	0·5 gm.
Magnesium sulphate	0·2 gm.
Ferric sulphate	trace
Egg albumen (dried)	0·15 gm.
Agar	15·0 gm.
Water	1,000·00 c.c.

Albumen is dissolved in *N.*/10 sodium hydroxide until neutral to phenolphthalein, then added to the warm mixture.

Standard Agar for Soil Bacteria (Thornton)

Potassium phosphate (K_2HPO_4)	1·0 gm.
Magnesium sulphate - -	0·2 gm.
Calcium chloride - - -	0·1 gm.
Sodium chloride - - -	0·1 gm.
Ferric chloride - - -	0·002 gm.
Potassium nitrate - - -	0·5 gm.
Asparagine - - - -	0·5 gm.
Mannitol - - - -	1·0 gm.
Water to - - - -	1,000·0 c.c.

Dissolve the potassium phosphate and nitrate and the asparagine in 500 c.c. of water. Then add the magnesium sulphate, calcium, sodium and ferric chlorides in that order at room temperature, from standard solutions. (See below.) Next add 20 gm. of agar, make up to 1,000 c.c. and dissolve at 100° C. The agar should be soaked in 0·05 per cent sulphuric acid at room temperature for 15 minutes, washed until acid free, then dried before using. Filter through layer of absorbent cotton wool or paper pulp 0·5 in. thick. Dissolve the mannitol in the filtrate, make up to 1,000 c.c. with boiling water, against a counterpoise flask containing 1,000 c.c. of water. Cool to 60° C., and adjust the *p*H to 7·4 against brom-thymol blue. Tube and sterilize for 15 minutes at 15 lb. pressure. Incubate at 20° C. for 10–12 days to test sterility.

Standard Solutions for Thornton's Agar

	Per 1,000 c.c.
Magnesium sulphate—4 gm. in 100 c.c. of solution	Take 5 c.c.
Calcium chloride—2 gm. in 100 c.c. of solution	Take 5 c.c.
Sodium chloride—2 gm. in 100 c.c. of solution	Take 5 c.c.
Ferric chloride—0·04 gm. in 100 c.c. of solution	Take 5 c.c.

Silica Jelly (Onslow Formula)

Weigh 20 gm. of water glass. Dilute with 100 c.c. of freshly boiled distilled water, free from carbon dioxide. Pour 75 c.c. of

this into a mixture of 25 c.c. hydrochloric acid with 75 c.c. of water. Dialyze (in parchment) for 3–4 hours in running water. Add a very little dilute nitric acid. Pour into a dish to set. Jelly forms in 2 hours.

Silica Gel Medium (Doryland)

In 500 c.c. water dissolve 8·40 gm. of sodium silicate and 24·00 gm. of potassium silicate. Dilute hydrochloric acid to a strength just a little more than that required to neutralize an equal volume of silicate solution. To the acid then add :

Magnesium sulphate	0·5 gm.
Calcium oxide	0·01 gm.
Ferrous sulphate	0·01 gm.
Manganese sulphate	0·01 gm.
Ammonium sulphate	1·0 gm.

Standardize this solution against the silicate solution, so that 1 c.c. of acid neutralizes 1 c.c. of silicates. Use methyl orange. Standardize acetic, sulphuric and phosphoric acids in the same way, with phenolphthalein.

Mix :

Hydrochloric acid	153·5 c.c.
Acetic acid	153·5 c.c.
Sulphuric acid	77·0 c.c.
Phosphoric acid	116·0 c.c.

1 c.c. of this mixture will neutralize 1 c.c. of mixed silicates. The acid mixture is placed in a sterile flask and connected to a self-filling burette. The silicate solution likewise. Fill the burettes, and leave standing for 2 hours to become sterile. Plug the overflow of the burettes with cotton wool. Run 5 c.c. of each fluid into a sterile Petri dish and mix by rotating the dish. Now add dextrose or other non-acid carbon source, in sterile aqueous solution, so as to give a 1 per cent concentration in the medium. Sets solid in about 5 minutes. About 80 per cent of organisms will grow on this medium.

For nitrate-nitrogen substitute nitric acid for acetic acid in the

acid mixture. Other carbon or nitrogen sources may be substituted if found necessary.

AUTOCLAVE TEMPERATURES AND PRESSURES

Temperatures		Atmospheres	Pressures	
° F.	° C.		Kgm./sq. cm.	lb./sq. in.
212	100	1·00	1·03	14·6
221	105	1·19	1·23	17·4
230	110	1·41	1·46	20·07
239	115	1·67	1·72	24·4
248	120	1·96	2·03	28·8
257	125	2·29	2·37	33·5
266	130	2·66	2·76	39·8
275	135	3·09	3·19	45·0

Cultivation of Water Moulds (Saprolegniales)

Class material can be readily obtained by immersing in pond water sterilized seeds, cut in half, of hemp or cress. Sporangia appear in about 4 days, sex organs in about 6 days. Cultures subsequently can be transferred to corn-meal agar (see p. 93).

CHAPTER X

SOLUTIONS FOR VOLUMETRIC ANALYSIS

THE range of volumetric analyses commonly carried out in botanical class-work is not very extensive, and the number of stock solutions which it is desirable to keep at hand is correspondingly small. The specialized worker in ecology or in plant physiology will, of course, make use of standard reference books which cover the whole field, for his particular needs. In this chapter it is the student or those in charge of laboratory classes for students, whom we have had in mind in selecting receipts. They will find here collected the chief types of solutions which are likely to be in daily use, and which it is often necessary to make up in considerable quantities. To these have been added a few special methods, not so generally familiar, which experience has shown may be sometimes valuable even in class-work.

It should be mentioned that in titration the choice of a proper indicator does not always receive the attention it deserves. The chart on p. 107 shows a choice of suitable indicators for any particular *p*H. This method is to be preferred to the use of a Universal Indicator, though this or the British Drug Houses "4–11" indicator may be used for determining the *p*H of soils in the field. The quality of indicator used is important in accurate work.

Choice of Indicators in Titration

(1) *Strong acid and strong base, or, weak acid and weak base.* As near true neutrality as possible. Use brom-thymol blue or phenol red.

(2) *Weak acid and strong base* (for example, such acids as acetic, oxalic, benzoic, etc.). Solution when neutralized will be in alkaline *p*H. Use phenolphthalein (*p*H 8·3–10).

(3) *Strong acid and weak base* (for example, ammonium hydroxide). Solution will finally be in acid *p*H. Use methyl orange (*p*H 2·9–4·6).

Hydrochloric Acid Normal *

Dilute 100 c.c. of strong acid to 1 litre with water. Titrate with standard borax solution or with standard sodium carbonate solution, and add the necessary water to make each c.c. contain 0·0365 gm. of acid.

Sulphuric Acid Normal *

Dilute 40 c.c. of strong acid to 1 litre with water, cooling carefully. Pour the acid into half the quantity of water, cool, and add the remainder. Titrate with standard sodium carbonate solution and add the necessary water.

Nitric Acid Normal *

Add 75 c.c. of strong acid to 1 litre of water. Titrate against standard sodium carbonate solution and add the necessary water.

Potassium Hydroxide Normal

Dissolve 70 gm. of potassium hydroxide in 1 litre of water, and titrate against standard hydrochloric acid and add the necessary quantity of water.

Sodium Hydroxide Normal

Dissolve 60 gm. of stick sodium hydroxide in 1 litre of water. Titrate with standard hydrochloric acid and add the necessary water.

Sodium Carbonate Normal

Heat 100 gm. of sodium carbonate to dull redness in a large porcelain dish until the weight becomes constant. Weigh out accurately the anhydrous sodium carbonate produced, and dissolve in such a quantity of water that each c.c. of solution contains 0·053 gm. of the carbonate or 53·00 gm. per litre.

* See also tables on p. 149.

Oxalic Acid Normal

Weigh out accurately 63·02 gm. of the pure acid and dissolve in enough water to make 1 litre. This solution may be used as a standard for checking other solutions.

Acetic Acid Normal

Weigh out 70 gm. of the glacial acid and dilute with 1,000 c.c. of water. Titrate against standard sodium hydroxide and add the necessary water. The standard solution should contain 60 gm. per litre.

Barium Hydroxide Normal

Weigh out 170 gm. of barium hydroxide and dissolve in 1 litre of water, together with about 3 gm. of barium chloride. Titrate against standard hydrochloric acid and add the necessary water.

Borax Solution Normal

Recrystallize some pure sodium diborate several times, to obtain a pure sample, and expose the crystals for some days under a large inverted funnel supported on corks. Weigh out the crystals accurately, and make up with water so that each c.c. of solution contains 0·191 gm. borax.

Potassium Permanganate *N.*/10

Weigh out 4 gm. of potassium permanganate and dissolve in 1 litre of water. Titrate with standard oxalic acid. Add the necessary water, so that each c.c. shall contain 0·00316 gm. of permanganate.

Potassium Dichromate *N.*/10

Dissolve in such a quantity of water that each c.c. shall contain 0·0049 gm. of dichromate or 4·904 gm. per litre.

Ammonium Oxalate *N.*/10

Dissolve 9 gm. of the pure salt in 1 litre of water. Titrate with potassium permanganate, and add the water necessary to make each c.c. contain 0·0062 gm. of ammonium oxalate.

Iodine *N.*/10

Weigh out 15 gm. of pure re-sublimed iodine, and dissolve in 1 litre of water containing 50 gm. potassium iodide. Make up the solution with sufficient water, so that each c.c. shall contain 0·0127 gm. of iodine or 12·69 gm. per litre.

Sodium Thiosulphate *N.*/10

Dissolve about 30 gm. of the pure crystalline salt in 1 litre of water and titrate with standard iodine solution.

Silver Nitrate *N.*/10

Weigh out accurately 17 gm. of silver nitrate and dissolve in 1 litre of water. The solution should contain 0·017 gm. of silver nitrate per c.c. or 16·99 gm. per litre.

Sodium Chloride *N.*/10

Weigh out accurately about 6·0 gm. of dry sodium chloride which has been prepared by precipitating a saturated solution of salt in strong hydrochloric acid. Dissolve in such a quantity of distilled water that each c.c. contains 0·00585 gm. of sodium chloride.

Methyl Orange

Dissolve 1 gm. in 1 litre of water for a general indicator.

Phenolphthalein

Dissolve about 15 gm. in 1 litre of 90 per cent alcohol, and keep the solution well stoppered.

Litmus Solution (1)

Commercial litmus consists of litmus mixed with chalk or gypsum. Mix about 25 gm. of the solid with 1 litre of water. Allow to stand in a warm place for several hours, shaking occasionally. Filter and add drops of dilute nitric acid until a purple

colour is obtained. Keep the solution in a bottle with a perforated stopper, so that air has free access to the solution.

Preparing Litmus Solution for Bacteriological Purposes (2)

Place 80 gm. granulated litmus in a mortar and grind it with 300 c.c. of 40 per cent alcohol. Decant into a 2-litre flask. Repeat with a fresh 300 c.c. of alcohol, and again with 400 c.c., making 1 litre in all. With the last lot of alcohol pour the remains of the solid litmus into the flask. Boil 1 minute. Stand overnight. Decant off extract, but do not filter. Make up to 1,000 c.c. with water. Add *N.*/1 hydrochloric acid drop by drop until the extract is purple. This takes about 20 c.c. per litre. Add about 2 per cent to the culture medium.

PREPARATION OF SOLUTIONS OF SOME INDICATORS FOR *p*H DETERMINATION (Sorensen)

Indicator	*p*H range	Colour change	Concentration	Amount required in drops for 10 c.c. of solution
Methyl violet 6*B*	0·1– 3·2	Yellow—violet	0·05% in water	3–8
Diphenylamine orange = Tropæolin OO	1·4– 2·6	Red—yellow	0·01% in water	3–8
Dimethyl-amino azobenzine	2·9– 4·0	Red—yellow	0·08% in 80% alcohol	5–10
Methyl orange	2·9– 4·0	Red—yellow	0·01% in water	3–5
Congo red	3·0– 5·0	Violet—blue—red	0·01% in water	3–5
Methyl red	4·2– 6·3	Red—yellow	0·02% in 60% alcohol	4–5
Litmus	4·5– 8·3	Blue—red		
Paranitrophenol	5·0– 7·0	Colourless—yellow	0·04% in 60% alcohol	10–20
Neutral red	6·8– 8·0	Red—yellow	0·01% in 50% alcohol	10–20
α-Naphthol phthalein	7·3– 8·7	Yellow—green—blue	0·04% in 60% alcohol	4–12
α-Naphthol orange = Tropæolin OOO	7·6– 8·9	Yellow—red	0·01% in water	4–10
Phenolphthalein	8·3–10·0	Colourless—red	0·05% in 50% alcohol	10–20
Thymol phthalein	9·3–10·5	Colourless—blue	0·04% in 50% alcohol	3–20
Resorcinol orange = Tropæolin O	11·1–12·7	Yellow—orange	0·01% in water	5–10

TABLE OF INDICATORS FOR *p*H (Clarke & Lubs)

Indicator	*p*H range	Colour change	Indicator solution		Concentration per 100 c.c.
			Water per 0·1 gm. of dye	*N.*/20 NaOH per 0·1 gm. of dye	
Cresol red (acid)	0·6–1·7	Red—yellow	500 c.c.	5·9 c.c.	0·02%
Thymol blue (acid)	1·2–2·3	Red—yellow	250 c.c.	4·7 c.c.	0·04%
Metacresol purple	1·2–2·5	Red—yellow	250 c.c.	5·9 c.c.	0·04%
Bromphenol blue	3·4–4·6	Yellow—blue	250 c.c.	4·1 c.c.	0·04%
Bromchlor phenol blue	3·5–4·6	Yellow—blue	250 c.c.	3·5 c.c.	0·04%
Methyl red - -	4·4–5·7	Red—yellow	500 c.c. (60% alcohol)	7·4 c.c.	0·02%
Bromcresol green	4·1–5·3	Yellow—blue	250 c.c.	3·4 c.c.	0·04%
Bromcresol purple	5·5–6·8	Yellow—purple	250 c.c.	4·1 c.c.	0·04%
Chlorphenol red	5·4–6·7	Yellow—red	250 c.c.	5·2 c.c.	0·04%
Bromthymol blue	6·5–7·7	Yellow—blue	250 c.c.	3·5 c.c.	0·04%
Phenol red - -	7·2–8·5	Yellow—red	500 c.c.	6·3 c.c.	0·02%
Cresol red (alk.)	7·5–8·7	Yellow—red	500 c.c.	5·9 c.c.	0·02%
Thymol blue (alk.)	8·3–9·5	Yellow—blue	250 c.c.	4·7 c.c.	0·04%

Reduce the 0·1 gm. of dye-stuff in an agate mortar, and, whilst reducing, add the prescribed amount of *N.*/20 sodium hydroxide. When the substance is completely dissolved, pour the neutral solution, without loss, into a flask. Add distilled water to one-quarter of final volume, heat on water bath at 80°–90° C. until perfect solution has taken place, cool, and dilute to prescribed volume.

Owing to instability of bromcresol purple in presence of alkali, place the weighed dye in a flask and fill to three-quarters of final volume with distilled water. Add the necessary *N.*/20 sodium hydroxide and heat, while stirring, to 80°–90° C.

CHAPTER XI

PHOTOGRAPHIC REAGENTS

PHOTOGRAPHY has now become an essential part of botanical work, whether for recording the appearance of plants and vegetation in the field or for recording microscopic preparations. The preparation of lantern slides is also necessary for teaching purposes. Whether in time lantern slides will be replaced by the cinematograph film is open to question; but there is no doubt that increasing use will be made of this in the future, and many botanical departments are already provided not only with their own cinematograph projectors, but also with instruments for recording cinematograph films of microscopic objects. The portraying of the life-cycles of microscopic objects by time-lapse cinematography will do much to clarify the student's mind about such matters.

The reagents necessary for botanical photography are relatively few, though it is important to point out that great care should be exercised in the selection of plates and developers for different kinds of work. A plate suitable for landscape photography is useless for copying line drawings, while the work of the most suitable plate may be ruined by the employment of the wrong developer.

Orthochromatic or panchromatic process plates are necessary for many types of work, and the selection of suitable light filters to give the maximum contrast or maximum detail in photomicrographic work needs careful consideration. (See p. 159.)

While contrast is desirable for some work, it is equally undesirable in others, lantern slides are quite as frequently exhibited showing too much contrast, producing a soot and milk effect, as having too little contrast. The selection of the right plate, the

correct developer and the proper printing material is necessary before a good result can be obtained, even if the exposure and time of development are correct.

Metol-Hydroquinone Developer

Metol	1 gm
Sodium sulphite	15 gm
Hydroquinone	4 gm
Sodium carbonate	15 gm
Water	1,250 c.c.

This is a good general developer for plates and films.

Metol-Hydroquinone for Negatives and Papers (Wall)

Metol	2·25 gm.
Sodium sulphite	45 gm.
Sodium carbonate	35 gm.
Hydroquinone	4·7 gm.
Water	1,000 c.c.

For negatives, dilute with equal quantity of water. For paper, use 1 part to 3 parts of water, and add 1 drop of 10 per cent potassium bromide to each 30 c.c. of diluted developer.

Marion's Developer

Metol	2·5 gm.
Hydroquinone	10·0 gm.
Potassium metabisulphite	2·5 gm.
Potassium bromide	1·5 gm.
Sodium sulphite	150·0 gm.
Sodium carbonate	150·0 gm.
Water	1,000·0 c.c.

For fine-grain panchromatic plates, dilute with 8 times its volume of water. For bromide papers dilute with 3 times its volume of water. For gas-light papers dilute with an equal volume of water.

Metol-Quinol Developer (Boyd)

Metol	14 gm.
Hydroquinone	56 gm.
Potassium bromide	12 gm.
Warm water	1,000 c.c.

When dissolved, add 400 gm. sodium sulphite, and to the white pasty mass add 40 gm. of sodium hydroxide.

For plates and gas-light paper use 1 part of stock solution to 7 parts of water.

For bromide paper and lantern slides use 1 part of stock solution to 15 parts of water, and add 2 drops of 10 per cent potassium bromide solution.

Metol-Hydroquinone Developer for Lantern Slides (Cramer)

Solution *A*	Metol	2 gm.
	Hydroquinone	6 gm.
	Sodium sulphite	30 gm.
	Water	750 c.c.
Solution *B*	Sodium carbonate	15 gm.
	Water	750 c.c.

Mix in equal parts. When fresh add 1 drop of 10 per cent potassium bromide to each 30 c.c. of solution.

Hydroquinone Developer

Solution *A*	Hydroquinone	20 gm.
	Sodium sulphite	100 gm.
	Citric acid	8 gm.
	Potassium bromide	5 gm.
	Water	1,000 c.c.
Solution *B*	Sodium hydroxide	20 gm.
	Water	1,000 c.c.

Use 100 c.c. each of *A* and *B* to 200 c.c. of water.

Pyro-Soda Developer for Negatives (Cramer)

Solution *A*	Sodium disulphite	4·5 gm.
	Pyrogallol	30 gm.
	Water	640 c.c.

Solution *B*	Sodium sulphite (anhyd.)	60 gm.
	Water - - - -	640 c.c.
Solution *C*	Sodium carbonate (anhyd.)	30 gm.
	Water - - - -	640 c.c.

Mix 1 part of each of solutions *A*, *B* and *C* with 8 parts of water.

Pyro-Soda Developer (Ilford)

Solution *A*	Sodium carbonate - -	100 gm.
	Sodium sulphite - -	100 gm.
	Potassium bromide -	2 gm.
	Water - - - -	1,000 c.c.
Solution *B*	Pyrogallol - - -	50 gm.
	Nitric acid - - -	20 drops
	Water - - - -	300 c.c.

Take 1 part of *B* and make up to 10 parts with water, and then add 10 parts of *A*. Solution *B* is better made up frequently.

Fine-grain Developer

Metol - - - - -	2·0 gm.
Hydroquinone - - - -	5·0 gm.
Sodium sulphite - - -	200·0 gm.
Borax - - - - -	8·0 gm.
Boric acid - - - -	8·0 gm.
Water - - - - -	1,000·0 c.c.

Use without dilution. Using Kodak Panatomic plates, develop for about 15 minutes at 19° C.

Fine-grain Developer (Kodak *D* 76)

Metol - - - - -	2 gm
Hydroquinone - - - -	5 gm
Sodium sulphite - - - -	200 gm
Borax - - - - -	2 gm
Water - - - - -	1,000 c.c.

Develop for 9–12 minutes.

Amidol Developer for Gas-light Papers

Sodium sulphite	25 gm.
Amidol	4 gm.
Potassium bromide 10 per cent aq. sol.	1 c.c.
Water to	500 c.c.

Dissolve in warm water in the order given and use when cold. When the temperature is high (70° F.) double the quantity of bromide, while at 80° F. use 6 times the quantity of bromide.

Developer for Process Plates

Solution *A*	Hydroquinone	22 gm.
	Sulphuric acid	2 c.c.
	Sodium sulphite	15 gm.
	Water	500 c.c.
Solution *B*	Sodium carbonate	15 gm.
	Potassium carbonate	45 gm.
	Potassium bromide	4 gm.
	Sodium sulphite	45 gm.
	Water	500 c.c.

For use mix equal parts of *A* and *B*.

Developer for Strong Contrast in Negatives and Lantern Slides

Hydroquinone	20 gm.
Sodium sulphite (anhyd.)	60 gm.
Sodium carbonate (anhyd.)	140 gm.
Potassium bromide	12 gm.
Water	1,000 c.c.

If kept in a tightly stoppered bottle, with no air space at the top, this developer will keep well. Otherwise it must be made up freshly.

Developer for Bromide Enlargements (Agfa)

Metol	1 gm.
Sodium sulphite	15 gm.
Hydroquinone	4 gm.
Sodium carbonate	15 gm.
Potassium bromide	1·8 gm.
Water	1,250 c.c.

Use without dilution and change frequently.

Developer for Line Work (Cramer)

Solution *A*	Hydroquinone - -	45 gm.
	Sodium sulphite - -	30 gm.
	Sulphuric acid - -	4 c.c.
	Water - - - -	1,000 c.c.
Solution *B*	Sodium carbonate -	30 gm.
	Potassium carbonate -	30 gm.
	Potassium bromide -	8 gm.
	Sodium sulphite - -	90 gm.
	Water - - - -	1,000 c.c.

Use equal quantities of solutions *A* and *B*. This developer gives a high degree of contrast.

Physical Development (Wratten)

Metol - - - - - -	5 gm.
Citric acid - - - - -	10 gm.
Glacial acetic acid - - - -	25 c.c.
Water - - - - - -	500 c.c.

This solution keeps well before silver is added. To the above solution add one-tenth of its volume of 10 per cent silver nitrate solution, that is, to $1\frac{1}{4}$ fluid oz. of developer add 1 dram of silver nitrate solution for each quarter-plate.

Density depends entirely on duration of development. Exposure should be about four times normal. Development may last from 2 to 5 minutes. The image goes back considerably in fixing, so that under-development is to be avoided. Any silver deposited on the negative can be wiped off after fixing with a pad of cotton wool.

Pinacryptol Green

A stock solution of 1 gm. of pinacryptol green in 500 c.c. of water is made and diluted 1 : 9 with water before use. To render panchromatic plates insensitive to red light, soak the plates in the solution for 2 minutes. Wash and then develop as for ordinary plates.

Fixing Solution

Sodium thiosulphate - - -	300 gm.
Water - - - - -	1,000 c.c.

An acid fixing bath may be made by the addition of 25 gm. of potassium metabisulphite to the above quantities. The solution should be diluted with an equal quantity of water for papers.

Acid Fixing Solution

Sodium thiosulphate - - -	150 gm.
Sodium bisulphite - - -	15 gm.
Water - - - - -	1,000 c.c.

Recommended for all kinds of plates.

Hypo Eliminator

Place the plates in water in a dish, and add slowly a solution of potassium permanganate till the pink colour remains unchanged. Then wash in water. If the quantity of potassium permanganate added is great it may act as a reducer, so that some preliminary washing is advisable before the potassium permanganate is used.

Hardening Bath

Formalin - - - - -	15 c.c.
Water - - - - -	150 c.c.

Soak plates or films for 10 minutes and then wash.

Mercury Intensifier

Solution *A*	Mercuric chloride - -	30 gm.
	Water (hot) - - -	500 c.c.

Pour off when cold and add 30 drops of hydrochloric acid.

Solution *B*	Ammonium hydroxide -	20 drops
	Water - - - -	30 c.c.

Wash plates free from hypo and soak in solution *A* until bleached. Blacken in solution *B*. Wash in running water for half an hour. Solution *A* may be used repeatedly.

Intensifier for Very Weak, 'Ghost' Negatives (Zelger)

Solution *A*	Copper sulphate - -	5 gm.
	Glacial acetic acid - -	28 c.c.
	Water - - - -	500 c.c.
Solution *B*	Potassium iodide - -	5 gm.
	Ammonia (67 per cent of 0·880) - - -	46 c.c.
	Water to make - -	250 c.c.

Bleach negative in a mixture of 2 parts *A* to 1 part *B*. Wash for 20 minutes in running water. Then darken with :

Silver nitrate - - - -	1·5 gm.
Sodium acetate - - - -	6·0 gm.
Water - - - - -	570·0 c.c.

If it is essential to avoid staining the negative, treat with alum solution between bleaching and darkening.

Chromium Intensifier

Potassium dichromate - -	23 gm.
Hydrochloric acid - - -	0·3 c.c.
Water - - - - -	1,000 c.c.

Bleach in above solution. Wash till free from yellow stain and redevelop in Amidol or other non-staining developer. Prolonged fixing is unnecessary, and the process may be repeated.

Lead Intensifier

This is only suitable for line subjects.

Lead nitrate - - - -	45 gm.
Potassium ferricyanide - -	68 gm.
Acetic acid - - - -	19 c.c.
Water - - - - -	1,000 c.c.

Wash carefully in 10 per cent nitric acid and then in water. Darken the negative in the following :

Sodium sulphide - - -	50 gm.
Water - - - - -	1,000 c.c.

or

Potassium dichromate	100 gm.
Ammonium hydroxide	50 c.c.
Water - - -	1,000 c.c.

Farmer's Reducer

Make a 1 in 5 solution of sodium thiosulphate, and add enough crystals of potassium ferricyanide to make it pale yellow. Negatives must be thoroughly soaked in water before being placed in the solution, and must be dropped into water as soon as reduction has proceeded far enough.

Persulphate Reducer

Ammonium persulphate - -	35 gm.
Sulphuric acid - - - -	18 drops
Water - - - - - -	1,000 c.c.

Use solution fresh. As soon as the negative is sufficiently reduced, immerse in 5 per cent sodium sulphate solution and then wash.

Colour Sensitizer for Plates

Pinacyanol stock solution - -	2 parts
Pinachrome stock solution - -	3 parts
Water - - - - - -	50 parts

The stock solutions are made by dissolving 1 part of the dye in 1,000 parts of water. The mixture should be freshly made, and not used more than twice. Immerse the plates for 3 minutes. Wash in running water for 5 minutes and dry. Plates treated in this way are sensitive to all colours (panchromatic).

Clearing Lantern Slides after Development

Alum - - - - -	20 gm.
Ferrous sulphate - - -	20 gm.
Citric acid - - - -	20 gm.
Water - - - - -	500 c.c.

This solution should be used after the slides come out of the fixing bath.

Gold Toning Solution

Gold chloride	0·3 gm.
Ammonium thiocyanate	3·5 gm.
Water	1,000 c.c.

Dissolve the gold chloride and the ammonium thiocyanate separately in hot water. Mix the two, stirring all the time. This quantity will tone thirty quarter-plate prints. Wash prints for 15 minutes in running water before toning. When the desired colour is obtained, wash, fix, and wash again for an hour at least.

Sepia Toning for Lantern Slides

Solution *A*	Metol	1·5 gm.
	Hydroquinone	6·0 gm.
	Sodium sulphite	50·0 gm.
	Sodium carbonate	100·0 gm.
	Potassium bromide	1·0 gm.
	Water	1,000·0 c.c.
Solution *B*	Ammonium carbonate	30 gm.
	Ammonium bromide	30 gm.
	Water	250 c.c.

Cold sepia tones: mix 8 parts of *A* to 1 part of *B*. Develop 5–8 minutes.

Warm sepia tones: mix 10 parts of *A* to 3 parts of *B*. Develop 12–15 minutes.

Purple-red sepia tones: mix 8 parts of *A* to 5 parts of *B*. Develop 20–30 minutes.

Rapid Drying Bath for Negatives

Soak the plate in two successive baths of 90 per cent alcohol, and then place in a current of air.

Lead Sulphide Ruling on Photographic Plates

Solution *A*	Lead acetate 10 per cent	100 c.c.
	Thiocarbamide 10 per cent	100 c.c.
	Water	100 c.c.
Solution *B*	Potassium hydroxide 10 per cent	250 c.c.

The two solutions are mixed before use and stirred, the plate is gently heated, placed in the liquid and rocked. The layer deposited on the glass enables very fine lines to be ruled in it.

Photographic Reproduction of Precision Scales

Clean glass plates are coated with a mixture of 90 c.c. egg albumen in 30 c.c. distilled water. Centrifuge to remove excess of albumen and dry without heat.

Sensitize with 5 per cent silver nitrate solution. Wash and dry.

Such plates require 20 minutes' exposure at 100 cm. from 20 amp arc lamp or about 2 hr. with a 1000-watt lamp. They are developed for 20 minutes in the following developer:

Pyrogallol	0·75 gm.
Citric acid	0·75 gm.
Nitric acid	1 drop
Water	100 c.c.

6 drops of 5 per cent silver nitrate are added to 20 c.c. of developer. This process yields a grainless image.

Removing Emulsion from Photographic Plates

The emulsion may be dissolved with 10–20 per cent sulphuric acid, and the slides when required can be cleaned with soap and water.

'Cellophane' for Lantern Slides

'Cellophane' cut to the size of a lantern plate is inserted in a folded sheet of carbon paper with the two carbon surfaces inside. The required legend is then typed, when the letters come out on both sides of the 'Cellophane' and give a good dense impression. 'Cellophane' may also be written on with indian ink. The sheets should be finally enclosed between glass-covers.

Varnish for Negatives

Celluloid	10 gm.
Amyl acetate	500 c.c.

To counteract the odour of amyl acetate a small proportion of oil of lavender may be added. The solution may be allowed to

flow over the negative, or may be applied with a brush to the cold negative.

Drawing on Lantern Slides

Lantern slide glasses may be coated by flowing over the surface a very thin solution of 0·25 gm. of Canada balsam in 50 c.c. of benzol. Dry by heat.

The surface takes indian ink well, and may also be painted upon with transparent oil colours thinned with turpentine. The latter should be applied in single strokes.

Ink Drawings from Prints

The following may be used on bromide or gas-light paper. After outlining the subject with waterproof indian ink, bleach the image with the following:

Thiocarbamide - - -	27·5 gm.
Nitric acid - - - -	25·0 c.c.
Water - - - - -	1,000·0 c.c.

Blocking-out Mixture

(1) Indian red water colour, students' quality in tubes, is good for blocking out. It should be thinned down for use, and does not crack.

(2) Commercial Brunswick black may be used, especially for large areas.

Sizes of Lantern Plates

English	3·25 in. × 3·25 in.
French	85 mm. × 100 mm. (long side horizontal)
American	4 in. × 3·25 in. (long side horizontal)

Cinematograph Films

Table indicating the rate of action, as seen on the screen, according to the rate at which the frames are exposed in the camera.

120 frames per second - - -	$\frac{1}{8}$ normal
64 frames per second - - -	$\frac{1}{4}$ normal

32 frames per second - - - .	$\frac{1}{2}$ normal
16 frames per second - - -	normal
8 frames per second - - -	2 normal
1 frame per second - - - -	16 normal
1 frame per minute - - - -	960 normal
1 frame per 4 minutes - - -	3,740 normal
1 frame per 6 minutes - - -	5,760 normal
1 frame per 10 minutes - - -	9,600 normal
1 frame per 15 minutes - - -	14,400 normal

Table of Relative Exposures in Copying

Relative size of copy	5	4	$3\frac{1}{2}$	3	$2\frac{1}{2}$	2	$1\frac{1}{2}$	1	$\frac{3}{4}$
Relative exposure	9	6	5	4	3	$2\frac{1}{2}$	$1\frac{1}{2}$	1	$\frac{3}{4}$
Relative size of copy	$\frac{2}{3}$	$\frac{1}{2}$	$\frac{1}{3}$	$\frac{1}{4}$	$\frac{1}{5}$	$\frac{1}{6}$	$\frac{1}{8}$	$\frac{1}{10}$	
Relative exposure	$\frac{3}{4}$	$\frac{3}{5}$	$\frac{2}{5}$	$\frac{2}{5}$	$\frac{2}{5}$	$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{3}$	

This table is intended for use with a copying camera, with fixed illumination. If the correct exposure for any size of copy is known, the table gives the correct exposure for any other size.

CHAPTER XII

WORKSHOP RECEIPTS

IT is remarkable how few botanical departments possess a well-equipped workshop. A bench in the preparation room is considered sufficient in many institutions, and no attempt is made either to repair or to construct apparatus. At the same time many pieces of apparatus can be easily made if attention is paid to this all-important section of botanical equipment. Much physiological apparatus which is sold at high prices can be constructed quite cheaply in the department workshop, while repairs can be quickly done, and the time wasted in sending apparatus back to the makers for minor adjustment may be saved. In the research laboratory new apparatus is constantly required, and the devising and constructing of equipment should form a part of the training of all postgraduate students.

The size and scope of a workshop will depend largely upon the funds available and the skill in making use of the tools provided. At the present time many small machine tools are available at reasonable prices which, even in the hands of an amateur, will soon save their cost in the improved work they will turn out and the time they will save. A botanical workshop should be equipped not only to deal with woodwork, but also with metal. A fairly wide range of wood tools should be provided, as well as the more commonly used metal ones. If electric power is available, a motor of about 1 horse-power may be installed, together with shafting from which various machine tools can be driven. The following machines will be found particularly useful: planing machine, 10-in. circular saw, drilling machine, $3\frac{3}{4}$-in. lathe, bandsaw and grinding machine. A hand morticing machine will also be found extremely useful.

It is impossible within the compass of this book to enter into the question of the workshop and its equipment at all fully. The following receipts will be found useful, and will also be found helpful to those who have no special workshop at their disposal.

Soldering Flux

Dissolve zinc scrap in concentrated hydrochloric acid. There should be excess of zinc.

Very good ready-made fluxes are 'Bakers' Preparation' or 'Fluxite'.

Metagelatine

20 per cent gelatine solution, heated with concentrated oxalic acid and then neutralized with chalk. Gelatine thus treated does not set when cold, but it retains its adhesive properties and makes excellent cold glue.

Luting Wax (See also p. 21)

Beeswax - - - - -	30 parts
Vaseline - - - - -	50 parts
Mix and add powdered resin - -	15 parts

The hardness of the mixture may be varied by increasing or decreasing the quantity of beeswax. This mixture is valuable for sealing joints in glass tubing.

Cleaning Mercury

Shake with a 1 per cent solution of mercurous nitrate, together with a few drops of strong nitric acid. Use equal volumes of solution and of mercury. Pour off the fluid and shake up with distilled water. Repeat the shaking with fresh solution and wash again with water. Filter through clean linen to take up surplus water, and dry by pouring through very fine needle holes in a filter paper contained in a filter funnel, or by heating to 110° C. in a dry oven.

Flame Proofing

A solution of ammonium sulphamate in water gives flame proofing to fabric and paper.

Blackening Laboratory Table-Tops (Spon)

Solution *A*	Ferrous sulphate - -	4 parts
	Copper sulphate - -	4 parts
	Potassium permanganate	8 parts
	Water - - -	100 parts

Boil to dissolve, and brush on two coats while hot, letting one coat dry before applying the second. Rub off excess and smooth thoroughly.

Solution *B*	Aniline oil - - -	12 parts
	Hydrochloric acid - -	18 parts
	Water - - - -	100 parts

Apply two coats as before, when a black colour will be obtained, impervious to the usual bench reagents.

Silvering Glass

Glass must be cleaned thoroughly with whiting paste, and polished off with a clean cloth when dry.

Solution *A* Silver nitrate 5 per cent aqueous solution. Add strong ammonium hydroxide, drop by drop, till the solution is *almost* clear. It should be slightly opalescent, that is, the precipitate must not be entirely dissolved.

Solution *B* Sodium potassium tartrate 5·7 gm. to 100 c.c. water.

Equal quantities of solutions *A* and *B* and of distilled water are mixed. Place in a dish and immerse the glass face downwards, just below the surface. Stand for 3–4 hours or overnight. The longer the time the denser the coat of silver deposited. Wash and dry thoroughly.

Fillers for Wood

(1) Plaster of Paris moistened with water immediately before use. The plaster should be tinted with a water stain to the final colour required before being used.

(2) Equal parts of dry whitening and of plaster of Paris moistened with turpentine to make a wet paste.

(3) *Beau Montage*. This is used for stopping, and is prepared by melting beeswax with an equal quantity of resin. Small quantities of shellac and of colouring matter are added. The mixture must be used hot.

(4) 'Plastic wood' filling—a proprietary article—may also be used.

Imperishable Putty

Spanish whiting	10 lb.
White lead	1 lb.
Boiled linseed oil	1 lb.
Olive oil	½ pint

Mix the Spanish whiting and white lead with the required amount of linseed oil, and then add the olive oil. Colouring matter may be added before mixing with the linseed oil. This putty does not harden.

To Prepare Lead Priming Paint

White lead	1 lb.
Patent driers	1 oz.
Red lead	½ oz.

Thin with a mixture of linseed oil and turpentine in the proportion of 2 : 1.

Preparing Size

Powdered size	2 oz.
Water	1 quart

Mix the size thoroughly with an equal volume of cold water, and then add the rest of the water, boiling. Apply before it is cold. A coat of size applied to woodwork before varnishing prevents the latter from soaking in. Stain may be added to the size, so that the colouring of the wood is done at the same time. Apply with a rag or large brush.

Vandyke Stain

A good general stain for wood may be made by dissolving, with heat, 4 oz. of powdered Vandyke brown in 1 pint of water.

Filter or decant off the liquid, and dilute to the required colour. Vandyke *crystals* will not dissolve in water.

Shellac Varnish for Polishing

Shellac - - - - - -	5 oz.
Alcohol 95 per cent - - -	1 pint

It will be ready to use in about 6 hours. The article to be polished must be dry and warm before beginning work.

Stain Colours for Wood

Mahogany. To make mahogany richer in colour use a solution of potassium dichromate in water. For darker results dissolve the dichromate in ammonia.

Walnut. To make a rich walnut colour dissolve equal quantities of powdered burnt umber and Vandyke brown in ammonia, and add water to give the right colour.

Oak. To darken oak, sponge with strong ammonia. Oak may be fumed by enclosing the article in an air-tight case with a saucer of strong ammonia (0·880).

Paint for Blackboards (1)

To make 1 gallon of paint take 10 oz. pulverized pumice stone ; 6 oz. pulverized rotten stone ; ¼ lb. lampblack, and mix with enough alcohol to make a thick paste. Grind the mixture thoroughly, and then dissolve 14 oz. shellac in the remainder of the gallon of alcohol. Stir together, and the paint is ready for use.

Paint for Blackboards (2)

Take a pint of alcohol and mix with it ½ lb. of unbleached shellac, and let it stand for 24 hours. To 2 pints of alcohol add ¼ lb. of lampblack and ¼ lb. flour of emery (powdered emery). Mix the two solutions thoroughly together. The paint should be of the consistency of varnish ; if too thick add alcohol, if too thin add lampblack.

Apply with as large a brush as possible.

If a plaster wall is to be used, sandpaper the wall and stop any cracks with plaster of Paris, then apply the above mixture.

Pulley Speed Calculations

Given	*Method*	*Result*
Diam. of driving pulley (a) Diam. of driven pulley (b) r.p.m. of driving pulley (c)	$\frac{a \times b}{c}$ =	r.p.m. of driven pulley (d)
Diam. of driving pulley (a) r.p.m. of driving pulley (c) r.p.m. of driven pulley (d)	$\frac{a \times c}{d}$ =	Diam. of driven pulley (b)
Diam. of driving pulley (a) Diam. of driven pulley (b) r.p.m. of driven pulley (d)	$\frac{b \times d}{a}$ =	r.p.m. of driving pulley (c)
Diam. of driven pulley (b) r.p.m. of driven pulley (d) r.p.m. of driving pulley (c)	$\frac{b \times d}{c}$ =	Diam. of driving pulley (a)

r.p.m. = revolutions per minute.

Duplicating Jelly (As for Hektograph)

Glycerine - - - - - -	6 lb.
Barium sulphate - - - -	2 lb.
Sheet gelatine - - - - -	1 lb.
Demerara sugar - - - - -	1 lb.

Soak all the materials in 4 pints of water for 24 hours. Put the whole in a saucepan and boil until the gelatine is dissolved, about an hour, stirring all the time. Pour into flat, level tin. Slightly warm the paper before drawing on it. Use a good smooth-surface paper for drawing, and press down very evenly on the jelly for 5 minutes, using a squeegee lightly. Peel off from one corner. Avoid any dragging movement of the paper. Fifty copies can be taken.

Accumulator Charging

Accumulators should be filled with sulphuric acid of specific gravity 1·250. A battery suffering from sulphation may be rectified by pouring out the acid, washing thoroughly with water, and then filling up with sodium sulphate 200 gm., water 1 litre

(¼ lb. to 1 pint). Charge for 60 hours. Wash out again with water, refill with fresh acid and recharge until gassing freely.

To Store Accumulators Safely

Charge fully and leave for about 6 hours. Pour off and keep the acid. Fill with distilled water and leave some hours. Pour off the water and screw up the filling cap tightly.

In this condition the accumulator will keep for months undamaged. To use again, fill with acid and recharge.

Grinding Glass

Use pure glycerine and emery powder.

Cutting Wide Glass Tubing

Scratch right round with a diamond. Wrap wet filter paper ½ in. wide round the tube to a thickness of about 2 mm. on both sides of the scratch and about 2 mm. from it. Heat the scratch in a very small flame while rotating the tube.

Polarity Paper

Add a little alcoholic phenolphthalein to a 10 per cent solution of potassium chloride in water. Soak filter paper in the solution and dry. For use moisten with water and touch with the battery leads. A red stain shows the negative terminal.

Lubricants for Cutting, Drilling or Tapping Metals

For steel, and most rolled metals, use the following :

Soft soap - - - - -	¼ lb.
Washing soda - - - -	1 oz.
Water - - - - - -	1 gallon

For cast metals, cut dry.

For copper and alloys rich in copper, cut dry, or for fine work, use milk.

For cool steel use turpentine.

For aluminium, cast and rolled, use turpentine or paraffin.

For tapping holes in steel use lard oil.

For tapping holes in cast iron, cut dry and finish off with white lead and tallow.

THREADS FOR MICROSCOPE AND CAMERA MOUNTS

R.M.S. and R.P.S. lens mounts	Outside diam., inches	Threads per inch	Remarks
Male	0·7982	36	Microscope obj. male
	0·7952	36	
Female	0·8030	36	Microscope obj. female
	0·8000	36	
1	1·0000	24	R.M.S. mounts
1	1·0000	32	Cine-lens mounts (U.S. standard)
1¼	1·2500	24	R.P.S. lens mounts
1⅜	1·3750	24	Ross lens mount, No. 0
1½	1·5000	24	R.P.S. and Ross lens mount, No. 1
1⅝	1·6250	24	Ross lens mount, No. 1a
1¾	1·7500	24	R.P.S. and Ross lens mount, No. 2
1⅞	1·8750	24	Ross lens mount, No. 2a
2	2·0000	24	R.P.S. and Ross lens mount, No. 3

SCREW THREADS OF B.A. FORMS

B.A. standard No.	Outside diam. in inches	No. of threads per inch	Pitch	Tapping size of drill; gauge No.	Driving fit size; gauge No.	Clearing size; gauge No.
16	0·0310	133·3	0·190	74	69	67
15	0·0350	120·5	0·210	72	66*b*	64
14	0·0390	109·9	0·230	70	62	1 m.m.
13	0·0470	102·0	0·250	65	56	55*f*
12	0·0510	90·9	0·280	62	56*b*	55
11	0·0590	81·9	0·310	56*f*	54	53
10	0·0670	72·6	0·350	55*f*	52*b*	50
9	0·0750	65·1	0·390	1·5 m.m.*f*	49*b*	48
8	0·0870	59·1	0·430	51*f*	45*b*	43
7	0·0980	52·9	0·480	48	41*b*	39
6	0·1100	47·9	0·530	44*f*	7/64	34
5	0·1260	43·0	0·590	40	1/8	30
4	0·1420	38·5	0·660	34	9/64	27
3	0·1610	34·8	0·730	30*f*	21*b*	19
2	0·1850	31·4	0·810	26	14*b*	3/16
1	0·2090	28·2	0·900	19	5*b*	3
0	0·2360	25·4	1·000	12	15/64	B

CHAPTER XIII

MISCELLANEOUS RECEIPTS

THE following receipts do not fall within the province of any of the previous chapters, but may be found of general use.

To soften Brittle Plant Material

Immerse the material for a week or longer in formol acetic alcohol. This may be made as follows :

Formalin - - - - -	6 c.c.
Acetic acid - - - - -	3 c.c.
Alcohol 50 per cent - - -	100 c.c.

Making Built-up Cells on Glass Slides (Hill)

Melt paraffin wax of about M.P. 52° C., and stir into it an equal quantity of dry powdered modelling clay or kaolin. This may be applied to the slide with a brush and turn-table. It adheres well to the glass, and forms hard, flat-topped walls which may be built up to any suitable height for moist chambers.

Special Hard Needles

Melt some sodium nitrate in a crucible. Take short lengths of tungsten wire of a suitable thickness and hold the tip in the molten mass ; this fines off the point and produces a needle with a very hard point. Such needles may be mounted in the usual handles.

Etching Glass

Glass may be etched by covering with hydrofluoric acid in a vessel coated with paraffin wax or in a gutta-percha or celluloid dish.

Solution for Cleaning Glass (1)

A strong solution of sodium metasilicate in water may be used for cleaning glassware. Old slides boiled in this mixture may be readily cleaned and the balsam dissolved quite easily.

Solution for Cleaning Glassware (2)

Sodium oleate - - - -	50 gm.
Tribasic sodium phosphate -	100 gm.
Water - - - - -	1,000 c.c.

Particularly useful for removing carbonaceous deposits.

Solution for Cleaning Microscope Slides (3)

The slides are placed in a hot 5 per cent solution of potassium dichromate, and a little strong sulphuric acid is added at intervals of 5–10 minutes. The mixture is kept bubbling for not less than 30 minutes. Slides are washed in running water for an hour or two, and kept in 95 per cent alcohol until required.

Cleaning Cover-Slips (4)

Cover-slips may be cleaned in 95 per cent alcohol, to which a few drops of hydrochloric acid have been added. They may be washed in 95 per cent alcohol, and stored in this until required. The slip is then taken out, dried by touching the edge with a piece of filter paper, and passed through a flame before being used.

Powerful Cleaning Solution (5)

Sulphuric acid - - -	1,000 c.c.
Potassium dichromate (sat. sol.)	100 c.c.

Will clean all glassware.

Chlorine Bleaching Solution for Plant Material or Sections

Potassium chlorate - - -	a few crystals
Hydrochloric acid - - -	1 drop
Alcohol 60 per cent - - -	100 c.c.

The spirit is added when a green colour indicates the evolution of chlorine. Prolonged washing is required after this reagent.

Bleaching Solution with Hydrogen Peroxide

Hydrogen peroxide (20 vols. solution)	40 c.c.
Alcohol 80 per cent - - - -	60 c.c.

Non-diathermic Solutions

Pure glycerine is the best non-diathermic fluid. A solution of a nickel salt, such as nickel sulphate, is also a most efficient shield against radiant heat. The traditional alum solution is of no value.

Standard Refractive Indices of Liquids

	N_D at 23° C
Methyl alcohol - - - -	1·3279
Water - - - - - -	1·3328
Acetone - - - - - -	1·3598
Chloroform - - - - -	1·4430
Trimethylene chloride - - -	1·4470
Glycerol - - - - - -	1·4671
Xylene - - - - - -	1·4957
Benzene - - - - - -	1·4982
Anisole - - - - - -	1·5150
Trimethylene bromide - - -	1·5220
Nitrobenzene - - - - -	1·5506
O-Toluidine - - - - -	1·5700
Aniline - - - - - -	1·5840
Bromoform - - - - -	1·5940
Cinnamaldehyde - - - -	1·6190
Quinolene - - - - -	1·6239
α-Bromonaphthalene - - -	1·6569
Methylene iodide - - - -	1·7400
Phenyl di-iodoarsine - - -	1·8430

Cobalt Paper for Transpiration Observations

Soak filter paper in 3 per cent cobalt chloride solution for several minutes. Hang up to drain. When air-dry transfer to oven at 100° C. until completely blue. Store in a bottle over calcium chloride.

Tincture of Iodine (Pharmaceutical)

Iodine	7 gm.
Potassium iodide	5 gm.
Water	5 c.c.

Dissolve and make up to 100 c.c. with absolute alcohol. Valuable for the sterilization of the skin or small wounds.

Synthetic Resin

Heat together equimolecular proportions of phthalic anhydride and ethylene glycol (respectively 2·4 parts to 1 part) for 24 hours at 200° C.

The resin is transparent and practically colourless. It sets hard and adheres well to glass, but softens at 95–100° C. Slowly dissolves in most organic solvents.

Celloidin Membranes; Preparation (Brown)

An 8 per cent solution of celloidin is made up in equal parts of ether and absolute alcohol. A test-tube of 1 cm. diameter is dipped into the solution. It is dried by insertion, inverted into the neck of an inverted ½-litre conical flask with a cork in the neck. Drain for 5 minutes, then immediately immerse in water. After 1 minute strip off the membrane from the test-tube by eversion. Wash and dry overnight at room temperature. The dry membrane should show a shrinkage of 10 ± 2 per cent.

Permeability is controlled by soaking in alcohol of varying concentrations for 24 hours. 95 per cent alcohol is the highest concentration which does not dissolve the membrane, and it gives it the greatest possible permeability. Pure water gives the lowest permeability.

The following are sample concentrations:

Membranes impermeable to: potassium permanganate <30 per cent alcohol; potassium oxalate <60 per cent; methylene blue <70 per cent; saponin <75 per cent; dextrose <85 per cent; litmus <92 per cent; Congo red <96 per cent.

After alcohol treatment transfer to distilled water and wash for one day. Membranes are then ready for use.

Membranes from 50–60 per cent alcohol are very efficient semi-permeable membranes and show strong endosmosis. A 90 per cent alcohol membrane retains starch, and can be used for the rapid dialytic separation of starch and dextrose.

Fossil Plant Strip Preparations, from Petrified Material (Walton)

After etching the cut surface of the block in dilute (about normal) hydrochloric acid, wash in water and allow to dry thoroughly.

The best films are cellulose ester-films made with a plasticiser, for example,

(Duerden)	Cellulose acetate - -	10 per cent
	Benzyl abietate - -	1 per cent
	Triacetin - - -	1 per cent

Dissolve in a mixture of acetone 4 parts to diacetone alcohol 1 part.

or (Graham)	20 sec. cellulose nitrate -	20 gm.
	Butyl acetate - -	200 c.c.
	Methyl phthalate - -	1 c.c.
	Xylol - - - -	10–20 c.c.

Leave for 24 hours before stripping.

Celluloid Film for Stomata (Long and Clements)

Standard solution:

Pyroxylin - - - - -	40 gm.
Ether - - - - - -	750 c.c.
Absolute alcohol - - - -	250 c.c.

Apply with a brush stroke and peel off when dry. More ether gives a tougher film, less ether gives a softer and more delicate film.

Ultra-Filtration (Ostwald's Quick Method)

Fold a coarse filter paper into a funnel and wet thoroughly. Fill with 4 per cent ether-alcohol colloidion solution. Pour out. Refill and again pour out. Fill with water to harden. Filters without pressure or suction. Will clarify most sols.

Bordeaux Mixtures

(1)	Copper sulphate - - -	12 lb.
	Lime - - - - -	8 lb.
	Water - - - - -	100 gallons

This solution is used as a spray for plants highly resistant to copper, such as grapes, potatoes and tomatoes.

(2)	Copper sulphate - - -	8 lb.
	Lime - - - - -	8 lb.
	Water - - - - -	100 gallons

This solution is used for fruit trees.

(3)	Copper sulphate - - -	4 lb.
	Lime - - - - -	4 lb.
	Water - - - - -	100 gallons

Occasionally used for plants with very sensitive foliage.

Spreader for Bordeaux Mixture

Add to the mixture 0·15 per cent of a potassium resin soap, which is prepared by boiling together the following:

Resin - - - -	2 parts
Potassium hydroxide -	1 part (sodium hydroxide will not do)
Water - - - -	3 parts

Improves the spreading power and adhesion of the spray.

Burgundy Mixture

Copper sulphate - - -	8 lb.
Washing soda - - - -	10 lb.
Water - - - - -	40 gallons

Liver of Sulphur

Potassium sulphide - - -	2 lb.
Water - - - - -	100 gallons

Some soft soap should be added to yield a soapy solution.

Lime Sulphur

The strong solution, of specific gravity 1·3, is difficult to prepare, and should be bought. It is diluted with 30 times its volume of water before use.

CHAPTER XIV

PHYSICAL AND CHEMICAL FORMULÆ AND EQUATIONS

THE botanist, and more particularly the plant physiologist, finds it necessary at times to make use of various chemical and physical constants, and to employ certain formulæ and equations in connexion with his work. It is impossible in the space available to do more than mention some of those most generally used. No attempt has been made to indicate their application.

N.T.P. Formula

For a dry gas $$V=\frac{v\times 273\times p}{760\times T}.$$

For a moist gas $$V=\frac{v\times 273\times (p-w)}{760\times T}.$$

V = corrected volume at normal temperature and pressure (0° C. and 760 mm.).

v = observed volume.

p = observed barometric pressure.

T = observed temperature on absolute scale, that is, thermometer reading (Centigrade) + 273.

w = vapour pressure of water at the observed temperature, in mm. mercury.

Standard Deviation of a Population of Observations

$$\sigma=\sqrt{\frac{\Sigma(x-\bar{x})^2}{n-1}},$$

where

σ = standard deviation

Σ = 'the sum of'

x = value of an individual in the population

$\bar{x}$ = mean of population

n = number of individuals.

Standard Error

$$\sigma/\sqrt{n}.$$

Where σ = standard deviation of the population and n is the number of individuals.

Determining Refractive Indices of Liquids with the Microscope.

Cement a ring on to a microscope slide and make a mark on the glass at the bottom of the chamber thus formed. Put on a cover-slip and focus the mark. Remove the slide without altering the microscope adjustments and fill the chamber with the liquid. Replace the slide on the stage and refocus the mark, observing the amount of vertical displacement of the microscope tube in doing so.

$$R.I. = \frac{b}{b-a}.$$

Where a = distance of change of focus
b = depth of the liquid in the chamber.

Sodium light should be used.

Resolution of Details under the Microscope (Airy)

$$X = 0{\cdot}5\, F\, \frac{L}{D}.$$

Where X = minimum separable distance between two structures
F = focal length of lens
D = diameter of lens
L = wave-length of light used in Ångstrom units.

Polarimetric Measures

To determine specific rotation.

$[\alpha]\,\frac{t}{D}$ at temperature t for D light (sodium).

Pure liquids. $[\alpha]\,\frac{t}{D} = \frac{a}{l\,d}$.

a = angle of rotation observed
l = length of tube in decimetres
d = density of liquid.

Solutions. $[\alpha]\frac{t}{D}=\frac{100\,a}{l\,c}$.

c=grams of active substance in 100 c.c. of solvent.

For determining concentration of a solution.

$$c=\frac{100\,a}{l\,r},$$

where r=specific rotation of the substance.

If the specific rotation is quoted for any other percentage than 100 per cent, the figure given is used in place of 100 in the formula.

Van't Hoff Coefficient of Temperature Effect (Q_{10})

$$Q_{10}=\Delta^T\sqrt{\left(\frac{V_2}{V_1}\right)}\ 10.$$

Δ^T=observed temperature difference
V_2=velocity at higher temperature
V_1=velocity at lower temperature.

Note. To find a higher root of a quantity, divide its logarithm by the required root.

Measurement of Surface Tension

(1) By means of the stalagmometer :

$$S.T.\ (\text{relative to water}=1{\cdot}0)=\frac{Nw}{N}D.$$

Nw=number of water drops formed
N=number of drops of test fluid formed
D=specific gravity of the test fluid.

(2) By means of the wire-ring method.

$$S.T.=\frac{Wg}{2C}\ \text{dynes},$$

W=weight required to break surface contact
g=gravitational constant C.G.S.=981 dynes
C=circumference of the wire ring.

3) By means of the capillary method.

Approximate formula :

$$\sigma = \frac{rhgD}{2}.$$

where σ = surface tension
r = radius of tube
h = height of rise
g = gravity constant = 981 dynes
D = density of liquid.

Viscosities

Viscosity of air at 10° C. = $172{\cdot}4 \times 10^{-6}$ dynes.
,, ,, ,, 20° C. = $178{\cdot}0 \times 10^{-6}$ dynes.

Relative viscosity of water at

0° C. = 1·000 coefficient = 0·0179 dynes,
15° C. = 0·637 ,, = 0·114 ,,
30° C. = 0·446 ,, = 0·008 ,,
100° C. = 0·158 ,, = 0·0028 ,,

Coefficient of viscosity of a liquid in comparison with water =

$$\frac{n_1}{n_2} = \frac{t_1 \times d_1}{t_2 \times d_2} \text{ dynes per sq. cm.,}$$

where n_1 = viscosity of water
n_2 = viscosity of liquid
t = time of flow in the viscometer
d = density of the liquid.

Weight-Volume Relationships

Air Specific gravity = 14·38 (H = 1).
Mean density of air = 0·0012.
1 gram = 773 c.c. at N.T.P.
1 c.c. = 0·00129 gm. at N.T.P.

Carbon dioxide Specific gravity = 1·5290 (air = 1).
1 gram = 505·8 c.c. at N.T.P.
1 c.c. = 0·00198 gm. at N.T.P.

Oxygen Specific gravity = 1·1053 (air = 1)
1 gram = 699·3 c.c. at N.T.P.
1 c.c. = 0·00142 gm. at N.T.P.

Water Density in gm. per c.c. at 0° C. = 0·999841,
at 3·98° C. = 0·999973,
at 15° C. = 0·999099.

At 15° C. 1 gm. water measures 1·00087 c.c.
At 3·98° C. 1 gm. water measures 1·00000 c.c.

Unit of Heat

It is equal to 1 gram-calorie.

Heat required to raise the temperature of 1 gm. of water by 1° C., i.e. from 15°–16° C.

Boiling-Point of Water

The boiling-point of water rises or falls 0·37° C. for a rise or fall of 10 mm. in the barometer.

Carrying Capacity of Electric Wires

Size S.W.G.	*Current-carrying capacity*
$\frac{3}{20}$ - - -	12·0 amp.
$\frac{7}{22}$ - - -	17·0 amp.
$\frac{7}{20}$ - - -	24·0 amp.
$\frac{7}{18}$ - - -	35·0 amp.

Electric-light branch circuits may be wired with $\frac{1}{18}$ or $\frac{3}{22}$ wire.

E.M.F. of Primary Cells

Cell	E.M.F.
Weston - - -	1·018 volts ; constant
Leclanché - -	1·47 volts ; falls rapidly
Bichromate - -	2·0 volts ; falls rapidly
Daniel - - -	1·1 volts ; constant
Bunsen - - -	1·9 volts ; constant
Grove - - -	1·7 volts ; constant

General Formula for Heat Production by an Electric Current

$$H=\frac{c^2Rt}{J} \text{ calories or } \frac{V^2t}{RJ},$$

where
c = current in amperes
R = resistance of circuit in ohms
t = time in seconds
J = Joule = 4·2
V = volts.

Electric Heater for Water

Nichrome strip $\frac{1}{32}$ in. × 0·004 in. (12 ohms to yard), London & Smith Wire Co.

Coil of $2\frac{1}{2}$ yd. connected through lamp bank to mains. Solder wire to copper terminals and wind on $\frac{1}{2}$ in. glass tube. Coat with insulating varnish. Lamp bank should have three 32-c.p. carbon lamps, in parallel.

Using 400 c.c. water:

0·5 amp.	gives rise of	2·2° C.	in	5	minutes
1·0 amp.	,, ,,	7·6° C.	,,	,,	
1·5 amp.	,, ,,	15·3° C.	,,	,,	

Simple Electrical Heater for Boiling Water

For 220-volt circuit. Use 40 ft. of 26 S.W.G. nichrome wire, wound on a thin asbestos former and insulated with asbestos or alundum cements.

Velocity of Light

= 2·9986 × 10^{10} cm. per second
= 186,330 miles per second.

Electric Lamps

Relation of candle-power and wattage

	C.P. per watt	Watts per C.P.
Carbon filament - -	@ 0·20	@ 5·0
Gas-filled - - -	0·6–1·6	0·6–1·5

Current in a lamp $=\frac{\text{watts}}{\text{voltage}}$, for example, $\frac{60}{230}=0{\cdot}26$ amp.

32 c.p. carbon filament = $32 \times 5 = 160$ watts at 230 volts
$= 0{\cdot}7$ amp.

Resistance of a lamp $= \frac{\text{volts}^2}{\text{watts}}$, for example, for 60-watt lamp on 230 volts = 882 ohms.

60-watt metal-filament lamp at 240 volts :

Resistance cold = 50 ohms ;
Resistance hot = 825 ohms.

32-c.p. carbon filament lamp at 240 volts :

Resistance cold = 680 ohms ;
Resistance hot = 416 ohms.

Light Units

1 foot candle = intensity at any point 1 ft. from a standard candle.

1 lux = 1 metre candle.

1 foot candle = 12·27 lux.

1 lumen = amount of light passing through an area R^2, where R is radius of a sphere with the light-source as centre. 4π lumens = 1 spherical candle.

1 watt = 668 lumen hours.

Full sunlight at noon on a clear summer day = about 6,800 foot candles or 83,436 lux.

Ultra-violet Light Filters

A filter for the 313 $\mu\mu$ line of the mercury arc can be prepared by making a warm-brown-toned lantern slide, which is very opaque to blue and violet light.

EQUIVALENCE OF PRACTICAL PHYSICAL UNITS

Henry

This is the unit of induction.

It equals 10^9 C.G.S. units, or the induction produced in a circuit when the induced E.M.F. is 1 volt and the inducing current varies at the rate of 1 ampère per second.

Farad

This is the unit of electrical capacity.

It equals 10^{-9} C.G.S. units, or the capacity of any condenser which requires 1 coulomb to raise its potential by 1 volt.

Coulomb

This is the unit of electrical quantity.

It equals 10^{-1} C.G.S. units, or the quantity which passes any point in 1 second in the case of a conductor carrying a current of 1 ampere.

Ohm

This is the unit of electrical resistance.

It equals 10^{9} C.G.S. units, or the resistance at 0° C. of a column of mercury 106·3 cm. long, of uniform cross-section and weighing 14·4521 gm.

Volt

This is the unit of electromotive force.

It equals 10^{8} C.G.S. units, or the force producing a current of 1 ampère through a resistance of 1 ohm.

Ampère

This is the unit of current.

It equals 10^{-1} C.G.S. units, or the current which deposits 0·001118 gm. of silver per second.

Watt

This is the unit of electric-power.

It equals the rate at which work is done by a current of 1 ampère falling through 1 volt.

1 watt equals 1 joule per second,

or 0·00134 horse-power,

or 0·001 kilowatt,

or 3·44 heat units per hour,

or 0·73 ft. lb. per second.

Kilowatt

1 kilowatt equals 1,000 watts,

or 1·34 horse-power,
or 2,656,400 ft. lb. per hour,
or 737·3 ft. lb. per second,
or 3,440 heat units per hour.

Kilowatt Hour

This is the Board of Trade unit of electrical energy, that is, the energy supplied when the product of ampères, volts and hours is 1,000.

1 kilowatt hour equals 1,000 watt hours,

or 1·34 horse-power hours,
or 3,600,000 joules,
or 3,440 heat units,
or 366,848 kilogrammeters.

Joule

1 joule equals 1 watt second,

or 0·00000278 kilowatt hours,
or 0·102 kilogrammeters,
or 0·00094 heat units,
or 0·73 ft. lb.

Horse-power

1 horse-power equals 746 watts,

or 0·746 kilowatts,
or 33,000 ft. lb. per minute,
or 2,580 heat units per hour,
or 43 heat units per minute,
or 0·71 heat units per second.

Kilogrammeter

1 kilogrammeter equals 7·23 ft. lb.,

or 0·00000366 horse-power hours,
or 0·00000272 kilowatt hours,
or 0·0092 heat units.

Foot Pound

1 foot pound equals 1·36 joules,
or 0·1383 kilogrammeters,
or 0·000000377 kilowatt hours,
or 0·00129 heat units,
or 0·0000005 horse-power hours.

Heat Unit

1 heat unit equals 1,048 watt seconds,
or 772 ft. lb.,
or 0·252 calories,
or 108 kilogrammeters,
or 0·000291 kilowatt hours.

Ohm's Law

$$R=\frac{E}{C},$$

where R = resistance in ohms
E = E.M.F. in volts
C = current in ampères.

Resistances in series:

$$R=\frac{E}{C}=r_1+r_2+r_3,\ldots$$

where r_1, r_2, r_3, etc., are the separate resistances.

Resistances in parallel:

$$\frac{I}{R}=\frac{1}{r_1}+\frac{1}{r_2}+\frac{1}{r_3}.$$

Comparison of Thermometer Scales (See also table on p. 149)

To convert Centigrade to Fahrenheit:
Multiply by $\frac{9}{5}$ or 1·8 and add 32.

To convert Fahrenheit to Centigrade:
Subtract 32 and multiply by $\frac{5}{9}$ or 0·55.

TABLES OF WEIGHTS AND MEASURES

(1) Measure of length

12 inches	= 1 foot	= 30·48 cm.
3 feet	= 1 yard	= 91·44 cm.
5·5 yards	= 1 pole	= 502·9 cm.

(2) Measure of Surface

144 square inches	= 1 square foot	= 929·0 sq. cm.
9 square feet	= 1 square yard	= 8,361·0 sq. cm.
30·25 square yards	= 1 square pole	= 252,906 sq. cm.

(3) Measure of Capacity : Solid Measure

1,728 cubic inches	= 1 cubic foot	= 28,310 c.c. (approx.)
27 cubic feet	= 1 cubic yard	= 764,500 c.c. (approx.)

(4) Measure of Capacity : Liquid Measure

4 gills	= 1 pint	= 568·3 c.c.
2 pints	= 1 quart	= 1,136 c.c.
4 quarts	= 1 gallon	= 4,546 c.c.

(5) Measure of Weight : Avoirdupois

437·5 grains	= 1 ounce	= 28·35 gm.
16 ounces	= 1 pound	= 453·6 gm.
28 pounds	= 1 quarter	= 12,700 gm.

(6) Measure of Weight : Troy

60 grains	= 1 drachm *	= 3·89 gm.
24 grains	= 1 pennyweight	= 1·56 gm.
20 pennyweight	= 1 ounce	= 31·10 gm.

(7) Apothecaries' Fluid Measure

60 minims	= 1 drachm * (℥1)	= 3·55 c.c.
8 drachms	= 1 ounce (℥1)	= 28·41 c.c.
20 ounces	= 1 pint (*O*1)	= 568·3 c.c.

* The only drachm now generally recognized is the *fluid* drachm, but where drachms *weight* are given in a formula it refers to the Troy (or old apothecaries') drachm of 3·89 gm.

EQUIVALENTS OF WEIGHTS AND MEASURES

1 metre = 39·37079 inches = 3·270 ft. = 1·094 yards.
1 litre = 61·02705 cub. in. = 0·035 cub. ft. = 1·760 pints.
1 gramme = 15·43235 grains = 0·032 troy oz. = 0·035 avoir. oz.
1 troy oz. = 31·10 grammes = 480 grains = 1·097 avoir. oz.
1 avoir. oz. = 28·35 grammes = 437·5 grains = 0·912 troy oz.
1 fluid oz. = 28·41 c.c.
1 minim = 0·059 c.c.
1 cm. = 0·39 in. 2·5 cm. = 1 in.
1 chain (surveyors') = 66 ft. or 100 links.
1 link = 7·92 in.
1 micron (μ) = 0·001 mm.
1 millimicron ($m\mu$) = 1×10^{-6} mm.
1 micromicron ($\mu\mu$) = 1×10^{-9} mm. or 0·01 A.
1 Ångstrom unit = 1×10^{-7} mm.
1 microgram (γ) = 0·001 mgm.
1 microlitre (λ) = 1×10^{-6} litre.
1 cubic inch = 16·387 c.c.
1000 millilitres = 1000·027 c.c.

Area of a circle = square of diameter × 0·79.
Area of a sphere = square of diameter × 3·14.
Area of a cylinder = diameter × length × 3·14.
Volume of sphere = $\frac{1}{6}$ cube of diameter × 3·14.
Volume of cylinder = square of diameter × length × 0·79.

Conversion factors

To convert :
Grains per fluid ounce to grammes per litre—multiply by 2·28.
Drachms per 10 ounces to c.c.'s per litre—multiply by 12·5.
Drachms per litre into grains per ounce—multiply by 0·437.
Grains (avoir.) into grammes—multiply by 0·0648.
Drachms (fluid) into c.c.'s—multiply by 3·55.
Ounces (fluid) into c.c.'s—multiply by 28·41.

TABLE OF ATOMIC WEIGHTS OF SOME COMMON ELEMENTS

Element	Symbol	Atomic weight	Density gm./c.c.	Valency
Aluminium	Al	26·97	2·69	3
Antimony	Sb	121·76	6·62	3, 5
Arsenic	As	74·91	5·73	3, 5
Barium	Ba	137·36	3·78	2
Bismuth	Bi	209·00	9·80	3, 5
Boron	B	10·82	2·53	3
Bromine	Br	79·916	3·12	1
Cadmium	Cd	112·41	8·65	2
Calcium	Ca	40·08	1·54	2
Carbon	C	12·01	3·52	4
Chlorine	Cl	35·47	0·00317	1
Chromium	Cr	52·01	6·92	3, 6
Cobalt	Co	58·94	8·71	2, 3
Copper	Cu	63·57	8·89	1, 2
Fluorine	F	19·00	0·00170	1
Gold	Au	197·2	19·29	3
Hydrogen	H	1·0081	0·000090	1
Iodine	I	126·92	4·94	1
Iron	Fe	55·84	7·90	2, 3
Lead	Pb	207·21	11·34	2
Lithium	Li	6·94	0·534	1
Magnesium	Mg	24·32	1·74	2
Manganese	Mn	54·93	7·42	2, 4
Mercury	Hg	200·61	13·546	1, 2
Nickel	Ni	58·69	8·90	2, 3
Nitrogen	N	14·008	0·00125	3, 5
Osmium	Os	190·20	22·5	6
Oxygen	O	16·00	0·00143	2
Palladium	Pd	106·7	12·16	2, 3
Phosphorus	P	30·18	1·83	3, 5
Platinum	Pt	195·23	21·37	2, 4
Potassium	K	39·096	0·87	1
Selenium	Se	79·96	4·80	2
Silicon	Si	28·06	2·42	4
Silver	Ag	107·88	10·49	1
Sodium	Na	22·997	0·97	1
Strontium	Sr	87·63	2·50	2
Sulphur	S	32·06	2·07	2
Tin	Sn	118·7	7·3	2, 4
Tungsten	W	183·92	18·6	4
Uranium	U	238·07	18·7	2, 3
Zinc	Zn	65·38	7·04	2

TABLE FOR THE CONVERSION OF DEGREES CENTIGRADE INTO DEGREES FAHRENHEIT AND VICE VERSA

Cent.	Fahr.	Cent.	Fahr.	Cent.	Fahr.	Cent.	Fahr.	Cent.	Fahr.	Cent.	Fahr.
0	32·0	17	62·6	34	93·2	51	123·8	68	154·4	85	185·0
1	33·8	18	64·4	35	95·0	52	125·6	69	156·2	86	186·8
2	35·6	19	66·2	36	96·8	53	127·4	70	158·0	87	188·6
3	37·4	20	68·0	37	98·6	54	129·2	71	159·8	88	190·4
4	39·2	21	69·8	38	100·4	55	131·0	72	161·6	89	192·2
5	41·0	22	71·6	39	102·2	56	132·8	73	163·4	90	194·0
6	42·8	23	73·4	40	104·0	57	134·6	74	165·2	91	195·8
7	44·6	24	75·2	41	105·8	58	136·4	75	167·0	92	197·6
8	46·4	25	77·0	42	107·6	59	138·2	76	168·8	93	199·4
9	48·2	26	78·8	43	109·4	60	140·0	77	170·6	94	201·2
10	50·0	27	80·6	44	111·2	61	141·8	78	172·4	95	203·0
11	51·8	28	82·4	45	113·0	62	143·6	79	174·2	96	204·8
12	53·6	29	84·2	46	114·8	63	145·4	80	176·0	97	206·6
13	55·4	30	86·0	47	116·6	64	147·2	81	177·8	98	208·4
14	57·2	31	87·8	48	118·4	65	149·0	82	179·6	99	210·2
15	59·0	32	89·6	49	120·2	66	150·8	83	181·4	100	212·0
16	60·8	33	91·4	50	122·0	67	152·6	84	183·2		

TABLE FOR DILUTION OF ACIDS

Sulphuric Acid		Hydrochloric Acid		Nitric Acid	
Per cent wt. of Acid	Sp. gr. at 20° C.	Per cent wt. of Acid	Sp. gr. at 20° C.	Per cent wt. of Acid	Sp. gr. at 20° C.
99	1·8401	37	1·19	100	1·520
90	1·820	35	1·18	99	1·515
80	1·732	31	1·16	90	1·492
70	1·618	30	1·152	80	1·460
60	1·504	20	1·100	70	1·420
50	1·399	10	1·050	60	1·372
40	1·307	5	1·024	50	1·316
30	1·222	3·65 (normal)	1·0175	40	1·252
20	1·144			30	1·184
10	1·068			20	1·117
5	1·034			10	1·057
4·9 (normal)	1·033			6·3 (normal)	1·035
				5	1·027

For instructions for the preparation of normal solutions of sulphuric, hydrochloric, nitric and acetic acids see pp. 119-120.

DENSITY OF SULPHURIC ACID AT CERTAIN TEMPERATURES

Sulphuric Acid	0° C.	25° C.	50° C.
10 per cent - -	1·077	1·068	1·052
20 „ „ - -	1·155	1·140	1·120
30 „ „ - -	1·235	1·218	1·200
40 „ „ - -	1·320	1·300	1·280
60 „ „ - -	1·820	1·495	1·475
70 „ „ - -	1·630	1·605	1·580

TABLE FOR DILUTING ALCOHOLS

From alcohol of the strength given in the top column it is required to make solutions of strengths shown in the left-hand column, e.g. 90 per cent alcohol to be made into 65 per cent alcohol. Under column marked 90 per cent and in line with 65 per cent, the number 41·5 is found; 41·5 is therefore the number of parts of water to be added to 100 parts of 90 per cent alcohol in order to make a solution of 65 per cent alcohol, etc.

Dilution desired	Dilution given								
	90%	85%	80%	75%	70%	65%	60%	55%	50%
85% -	6·6								
80% -	13·8	6·8							
75% -	21·9	14·5	7·2						
70% -	31·1	23·1	15·1	7·6					
65% -	41·5	33·0	24·7	16·4	8·2				
60% -	53·7	44·5	35·4	26·5	17·6	8·8			
55% -	67·9	57·9	48·1	38·3	28·6	19·0	9·5		
50% -	84·7	73·9	63·0	52·4	41·7	31·3	20·5	10·4	
45% -	105·3	93·3	81·4	69·5	57·8	46·1	34·5	22·9	11·4
40% -	130·8	117·3	104·0	90·8	77·6	64·5	51·4	38·5	25·6
35% -	163·3	148·0	132·9	117·8	102·8	87·9	73·1	58·3	43·6
30% -	206·2	188·6	171·1	153·6	136·4	118·9	101·7	84·5	67·5
25% -	266·1	245·2	224·3	203·5	182·8	162·2	141·7	121·2	100·7
20% -	355·8	329·8	304·0	278·3	252·0	227·0	201·4	176·0	150·6
15% -	505·3	471·0	436·9	402·8	368·8	334·9	301·1	267·3	233·5
10% -	804·5	753·7	702·9	652·2	601·6	551·1	500·6	450·2	399·9

PROPERTIES OF CERTAIN SUGARS

Sugar	$[\alpha]_d$ at 20° C.	M.P. of ozazone
Arabinose - -	−105	218° C.
Xylose - -	+ 19·13	160° C.
Rhamnose - -	+ 8·07	180° C.
d-glucose - -	+ 52·5	205° C.
l-glucose - -	− 51·4	
Lævulose - -	− 88·5	158° C.
Galatose - -	+ 83·8	159° C.
Mannose - -	+ 14·1	195–200° C.
Sucrose - -	+ 66·41	
Maltose - -	+138·48	206° C.

CONDUCTIVITY OF STANDARD SOLUTIONS

Potassium chloride, normal solution, 74·59 gm. per litre of solution at 18° C.

Conductivity in reciprocal ohms.

Temp. ° C.	KCl normal	KCl $\frac{1}{10}$ *N.*	KCl $\frac{1}{100}$ *N.*
0 - -	0·06541	0·00715	0·000776
5 - -	0·07414	0·00822	0·000896
10 - -	0·08319	0·00933	0·001020
15 - -	0·09252	0·01048	0·001147
16 - -	0·09441	0·01072	0·001173
17 - -	0·09631	0·01095	0·001199
18 - -	0·09822	0·01119	0·001225
19 - -	0·10014	0·01143	0·001251
20 - -	0·10207	0·01167	0·001278
21 - -	0·10400	0·01191	0·001305
22 - -	0·10594	0·01215	0·001332
23 - -	0·10789	0·01239	0·001359
24 - -	0·10984	0·01264	0·001386
25 - -	0·11180	0·01288	0·001413
26 - -	0·11377	0·01313	0·002819
27 - -	0·11574	0·01337	0·002873
28 - -	——	0·01362	0·002927
29 - -	——	0·01387	0·002981
30 - -	——	0·01412	0·003036

FREEZING MIXTURES

Substance	*A*	*B*	Initial temp.	Temp. attained by mixture
Sodium nitrate - -	75	Water 100	+13·2° C.	- 5·3° C.
Calcium chloride -	250	Water 100	+10·8	-12·4
Ammonium nitrate -	60	Water 100	+13·6	-13·6
Sodium chloride - -	33	Snow 100	- 1	-21·3
Sulphuric acid 66·1% -	1	Snow 0·61	0	-39·0
Sulphuric acid 66·1% -	1	Snow 0·70	0	-54·9
Calcium chloride -	1	Snow 0·81	0	-40·3
Ammonium nitrate -	1	Water 0·94	+20	- 4·0
	1	Water 1·31	+10	-17·5

A = proportion of substance named in first column. This is added to the proportion of substance given in column *B*.

CONSTANT TEMPERATURE BATHS

Substance	Boiling-point at 760 mm. barometric pressure
Acetone - - -	56·1° C.
Methyl alcohol -	64·5
Ethyl alcohol - -	78·5
Water - - -	100·0
Toluene - - -	110·5
Iso-amyl acetate -	142·5
Aniline - - -	184·4

Lower boiling temperatures may be obtained by using reduced pressures.

VAPOUR PRESSURE OF WATER

In mm. of mercury

Temp. ° C.	V.P.	Temp. ° C.	V.P.	Temp. ° C.	V.P.
0·0	4·6	10·0	9·2	20·0	17·4
1·0	4·9	11·0	9·8	21·0	18·5
2·0	5·3	12·0	10·5	22·0	19·7
3·0	5·7	13·0	11·2	23·0	20·9
4·0	6·1	14·0	11·9	24·0	22·2
5·0	6·5	15·0	12·7	25·0	23·5
6·0	7·0	16·0	13·5	26·0	25·0
7·0	7·5	17·0	14·4	27·0	26·5
8·0	8·0	18·0	15·4	28·0	28·1
9·0	8·6	19·0	16·3	29·0	29·8

RELATIVE HUMIDITY

Conversion of readings of wet- and dry-bulb thermometer

Temp. °C.	Depression of wet-bulb thermometer in degrees C.									
	1·0	2·0	3·0	4·0	5·0	6·0	7·0	8·0	9·0	10·0
0	81	64	46	29	13					
+ 3	84	69	54	40	25	12				
+ 6	87	73	60	47	35	23	11			
+ 9	88	76	65	53	42	32	22	12		
+12	89	78	68	58	48	38	30	21	12	4
+15	90	80	71	62	53	44	36	28	20	13
+18	90	82	73	65	57	49	42	35	27	20
+21	91	83	75	67	60	53	46	39	32	26
+24	92	85	77	70	63	56	49	43	37	31
+27	93	86	79	72	65	59	53	47	41	36
+30	93	86	79	73	67	61	55	50	44	39

HUMIDITY CONTROL BY SULPHURIC ACID MIXTURES

Relative Humidity of Air which is in equilibrium with Sulphuric Acid of given strength

Sulphuric Acid per cent	0° C.	25° C.	50° C.
	R.H.	R.H.	R.H.
0 - -	100·0	100·0	100·0
5 - -	98·4	98·5	98·5
10 - -	95·9	96·1	96·3
15 - -	92·4	92·9	93·4
20 - -	87·8	88·5	89·3
25 - -	81·7	82·9	84·0
30 - -	73·8	75·6	77·2
35 - -	64·6	66·8	68·9
40 - -	54·2	56·8	59·3
45 - -	44·0	46·8	49·5
50 - -	33·6	36·8	39·9
55 - -	23·5	26·8	30·0
60 - -	14·6	17·2	20·0
65 - -	7·8	9·8	12·0
70 - -	3·9	5·2	6·7
75 - -	1·6	2·3	3·2
80 - -	0·5	0·8	1·2

STRENGTH OF SULPHURIC ACID REQUIRED TO GIVE DEFINITE RELATIVE HUMIDITY OF AIR

Rel. humidity per cent	Per cent Sulphuric Acid		
	0° C.	25° C.	50° C.
10 - -	63·1	64·8	66·6
25 - -	54·3	55·9	57·5
35 - -	49·4	50·9	52·5
50 - -	42·1	43·4	44·8
65 - -	34·8	36·0	37·1
75 - -	29·4	30·4	31·4
90 - -	17·8	18·5	19·2

OSMOTIC PRESSURES OF SUCROSE SOLUTIONS IN ATMOSPHERES

(Ursprung and Blum)

Molar conc. of solution -	0·01	0·05	0·10	0·15	0·20	0·25	0·30
Osmotic pressure - -	0·25	1·45	2·65	3·92	5·15	6·37	7·13

SOLUBILITY COEFFICIENTS OF OXYGEN AND CARBON DIOXIDE IN WATER

Volume in c.c. dissolved in 1 c.c. of water at given temperature and at pressure of 1 atmosphere, calculated to 0° C.

Temperature ° C.	Oxygen	Carbon dioxide
0 - - -	0·0489	1·713
5 - - -	0·0429	1·424
10 - - -	0·0380	1·194
15 - - -	0·0342	1·019
20 - - -	0·0310	0·878
25 - - -	0·0283	0·759
30 - - -	0·0261	0·665
40 - - -	0·0231	0·530
50 - - -	0·0209	0·436

SURFACE TENSION OF WATER

(In dynes per sq. cm.)

Temp.	S.T.	Temp.	S.T.
0° C.	75·6	40° C.	70·0
5° C.	74·9	50° C.	68·6
10° C.	74·2	60° C.	67·1
15° C.	73·5	70° C.	65·7
20° C.	72·8	80° C.	64·3
25° C.	72·1	90° C.	62·9
30° C.	71·4	100° C.	61·5
35° C.	70·7		

SURFACE TENSION OF ETHYL ALCOHOL PERCENTAGES

(In dynes per sq. cm. at 15° C.)

Alcohol	S.T.	Alcohol	S.T.
10%	51·2	60%	27·7
20	40·6	70	26·6
30	34·7	80	25·4
40	31·2	90	24·1
50	29·1	100	22·5

SORENSEN PHOSPHATE BUFFER MIXTURES

*p*H	c.c. basic salt	c.c. acid salt
6·0	1·4	8·6
6·2	2·0	8·0
6·4	3·0	7·0
6·6	4·0	6·0
6·8	5·0	5·0
7·0	6·1	3·9
7·2	7·0	3·0
7·4	7·8	2·2
7·6	8·5	1·5
7·8	9·1	0·9

Basic salt = *M.*/15 solution of dibasic sodium phosphate (Na_2HPO_4 $2H_2O$) containing 11·876 gm. in 1 litre of solution.

Acid salt = *M.*/15 solution of monobasic potassium phosphate (KH_2PO_4) containing 9·078 gm. in 1 litre of solution.

McILVAINE'S STANDARD BUFFER SOLUTIONS

Stock solution *A* : 0·1 molar citric acid solution.

Stock solution *B* : 0·2 molar disodium phosphate solution.

*p*H	Solution *A* c.c.	Solution *B* c.c.
5·0	9·70	10·30
5·2	9·28	10·72
5·4	8·85	11·15
5·6	8·40	11·60
5·8	7·91	12·09
6·0	7·37	12·63
6·2	6·78	13·22
6·4	6·15	13·85
6·6	5·45	14·55
6·8	4·55	15·45
7·0	3·53	16·47
7·2	2·61	17·39
7·4	1·83	18·17
7·6	1·27	18·73
7·8	0·85	19·15
8·0	0·55	19·45

GILLESPIE-HATFIELD TWO-TUBE METHOD OF MEASURING *p*H OF SOLUTIONS

Buffer Solutions

Solution *A* : 7·0 gm. potassium dihydrogen phosphate (KH_2PO_4) per litre.

Solution *B* : 1·0 gm. sodium carbonate (anhyd.) per litre.

Indicator : brom-thymol blue.

*p*H	Acid tube		Basic tube	
	c.c. indicator	c.c. phosphate	c.c. indicator	c.c. carbonate
6·15	0·10	10·90	0·90	10·10
6·35	0·15	10·85	0·85	10·15
6·5	0·20	10·80	0·80	10·20
6·7	0·30	10·70	0·70	10·30
6·9	0·40	10·60	0·60	10·40
7·1	0·50	10·50	0·50	10·50
7·3	0·60	10·40	0·40	10·60
7·5	0·70	10·30	0·30	10·70
7·7	0·80	10·20	0·20	10·80
7·85	0·85	10·15	0·15	10·85
8·05	0·90	10·10	0·10	10·90

Place 10 c.c. of the sample in one tube with 1 c.c. indicator and view through it and a plain water tube, in comparison with two tubes containing the two indicators and buffers, as given in the table. Standards are stable for 4 to 8 weeks.

IMPERIAL STANDARD WIRE-GAUGE AND ELECTRICAL RESISTANCE OF WIRES

Wire gauge number	Diameter mm.	Copper ohms per metre	Eureka & Constantan ohms per metre	German silver ohms per metre	Manganin ohms per metre
10	3·251	0·0021	0·057	0·027	0·051
12	2·642	0·0032	0·086	0·041	0·077
14	2·032	0·0054	0·146	0·070	0·131
16	1·626	0·0083	0·228	0·109	0·204
18	1·219	0·0148	0·405	0·193	0·361
20	0·9144	0·0260	0·722	0·345	0·645
22	0·7112	0·0435	1·20	0·57	1·07
24	0·5588	0·0705	1·93	0·92	1·73
26	0·4572	0·105	2·89	1·38	2·58
28	0·3759	0·155	4·27	2·02	3·82
30	0·3150	0·222	6·08	2·90	5·45
32	0·2743	0·293	8·02	3·83	7·18
34	0·2337	0·404	11·1	5·27	9·90
36	0·1930	0·590	16·2	7·74	14·5
38	0·1524	0·950	26·0	12·4	23·2
40	0·1219	1·48	40·6	19·4	36·3

TABLE OF PHOTOGRAPHIC ENLARGEMENT

Focus of lens in inches	Times of enlargement								
	1	2	3	4	5	6	7	8	
2	4	6	8	10	12	14	16	18	Camera extension in inches
3	6	9	12	15	18	21	24	27	
4	8	12	16	20	24	28	32	36	
5	10	15	20	25	30	35	40	45	

The distance between lens and object for any enlargement is obtained by dividing the camera extension by the enlargement.

WAVE-LENGTHS OF THE FRAUNHOFER LINES

Line	Wave-length	Line	Wave-length
U	2947·9	*G*	4307·914
t	2994·4	*G'*	4340·477
T	3021·067	*F*	4861·344
s	3047·623	b_4	5167·510
S	3100·683	b_2	5172·700
R	3181·277	b_1	5183·621
Q	3286·773	E_2	5269·557
P	3361·194	D_2	5889·977
O	3441·020	D_1	5895·944
N	3581·210	*C*	6562·816
M	3727·636	*B*	6869·955
L	3820·438	A_1	7621
K	3933·684	A_2	7594
H	3968·494	*Z*	8228·5
h	4101·750	*Y*	8990·0
g	4226·742		

LIGHT FILTERS

Wratten '*M*' filters (Kodak Ltd.)

Name of filter	Visual colour	Spectrum transmission
A	Scarlet	From red end to 5,900
B	Green	From 6,000 to 4,600
C	Blue-violet	From 5,100 to 4,000
D	Purple	From red end to 6,400 and 4,600 to 3,800
E	Orange	From red end to 5,600
F	Pure red	From red end to 6,100
G	Strong yellow	From red end to 5,100
H	Blue	From 5,400 to 4,200
K_3	Bright yellow	Luminosity screen for orthochromatic reproduction

MULTIPLYING FACTORS FOR WRATTEN FILTERS

Filter	K_1	K_2	K_3	*A*	*B*	*C*	*F*	*G*
Panchromatic plate	1½	3	4½	12	14	10	25	5
Orthochromatic plate	3	6	12	..	24	8	..	24

COMBINATION OF TWO FILTERS

Name of filter	Visual colour	Spectrum transmission
A and *D* -	Deep red	From red end to 6,400
A and *B* -	Dark brown	From 5,800 to 6,000
B and *E* -	Yellow-green	From 5,600 to 6,000
G and *H* -	Pure green	From 5,100 to 5,400
B and *C* -	Blue-green	From 4,600 to 5,100
D and *H* -	Violet	From 4,200 to 4,600

SPECTRUM ABSORPTION OF THE CHIEF HISTOLOGICAL STAINS IN COMPARISON WITH THAT OF '*M*' FILTERS SHOWING MAXIMUM CONTRAST

Stain	Spectral absorption bands	Screen	Band used
Aniline blue - - -	55–62	*B* and *E*	56–60
Bismarck brown - -	General in blue	*C*	40–51
Congo red - - -	48–52	*B* and *C*	46–51
Eosine - - - -	49–53	*G* and *H*	51–54
Erythrosine - - -	51–54	*G* and *H*	51–54
Fuchsine - - -	53–57	*B* and *G*	51–60
Gentian violet - - -	57–60	*B* and *E*	56–60
Hæmatoxylin (Ehrlich) -	Gradual through green	*B* and *G*	51–60
Hæmatoxylin (Heidenhain)	56–60	*B* and *E*	56–60
Iodine green - - -	62–65	*F*	61–68
Methylene blue - -	60–62 and 65–68	*D* and *G*	64–68
Methyl violet - - -	58–60	*B* and *E*	56–60
Methyl green - - -	62–65	*F*	61–68
Picro-carmine - - -	51–53 and 56–57	*G* and *H*	51–54
Rose Bengal - - -	53–56	*G* and *H*	51–54

For fossil sections use *C* screen for contrast and *F* screen for detail in sections.

APPENDIX I

LIST OF REAGENTS FOR MICROSCOPICAL WORK

Acetic acid, glacial
Acetone
Acid fuchsine = magenta, acid
Alcohol
Aluminium potassium sulphate = alum
Ammonium hydroxide
Aniline blue = spirit blue or soluble blue
Aniline oil
Arsenious acid
Azo blue

Basic fuchsine = magenta, basic
Benzene
Benzyl alcohol
Benzyl violet
Bergamot oil
Bismarck brown
Borax = sodium diborate
n-Butyl alcohol

Cadmium chloride
Canada balsam
Cane sugar = sucrose
Carbolic acid = phenol
Carmine
Carminic acid
Cedar-wood oil
Chloral hydrate
Chloroform
Chromic acid = chromic anhydride
Chrysoidin
Clove oil
Cochineal
Congo red
Copper acetate
Cotton blue = soluble blue
Crystal violet
Cyanine

Dahlia
Diethylene glycol

Eosine
Erythrosine
Ethyl phthalate
Euparal

Ferric ammonium sulphate
Ferric chloride
Ferric hydrate
Ferrous sulphate
Formalin = formaldehyde solution
Formic acid

Gelatine
Gentian violet
Glycerine jelly
Gold orange
Gum acacia
Gum dammar
Gum mastic

Hæmatoxylin
Hoffman's blue

Hydrochloric acid
Hyrax

Iodine
Iodine green
Iron alum = ferrous aluminium sulphate
Isopropyl alcohol

Lactic acid
Lithium carbonate
Light green

Magdala red
Magenta, acid
Magenta, basic
Malachite green
Martius yellow
Mercuric chloride
Methyl alcohol
Methyl blue = soluble blue
Methylene blue
Methyl green
Methyl violet = benzyl violet
Murrayite

Neutral red
Nigrosine
Nitric acid

Osmic acid = osmium tetroxide
Orange *G*
Orange tannin
Oxalic acid

Phenol
Picric acid
Platinic chloride
Polychrome blue
Potassium chlorate
Potassium dichromate
Potassium hydroxide
Potassium iodide
Potassium permanganate

Ruthenium red

Saponin
Safranine
Sodium bisulphite
Sodium carbonate
Sodium chloride
Sodium diborate
Sodium hydroxide
Sodium metabisulphite
Soluble blue
Spirit blue
Styrax
Sucrose
Sulphuric acid
Synthol

Tannin
Terpineol
Thionin
Thymol
Trichlorethylene
Turpentine oil

Urea

Venetian turpentine

Water blue

Xylol

Zinc iodate
Zinc phenolsulphonate

LIST OF BENCH REAGENTS

Glycerine 30 per cent
Lactophenol
Distilled water
Alcohol 50 and 70 per cent (see p. 174)
Iodine in potassium iodide
Aniline chloride (*or* sulphate)
Absolute alcohol (see p. 16)
Xylol (*or* clove oil *or* terpineol)
Canada balsam (*or* Euparal)
Basic stain } see p. 33
Acid stain }
Eau de Javelle

A set of these reagents is generally required for each student working on microscopical investigations.

APPENDIX II

LIST OF CHEMICALS REQUIRED FOR ELEMENTARY BIOCHEMICAL WORK

Acetic acid
Acetone
Agar
Alcohol
Albumen
Aluminium potassium sulphate = alum
Ammonium carbonate
Ammonium chloride
Ammonium hydroxide
Ammonium lactate
Ammonium nitrate
Ammonium oxalate
Ammonium phosphate
Ammonium sulphate
Ammonium tartrate
Aniline chloride
Aniline sulphate
Aniline oil
Asparagine

Barium chloride
Barium hydroxide
Basic fuchsine = magenta, basic
Benzidine
Borax = sodium diborate

Calcium chlorate
Calcium chloride
Calcium carbonate
Calcium hydrogen phosphate
Calcium nitrate
Calcium phosphate
Calcium sulphate
Camphor
Cane sugar = sucrose
Chloral hydrate
Chromic acid
Congo red
Copper acetate
Copper carbonate
Copper sulphate
Corn meal
Crystal violet

Dextrose
Dimethylamine
Dimethylamido azobenzene
Diphenylamine

Ether

Ferric ammonia sulphate
Ferric chloride
Ferric oxide
Ferric sulphate
Ferrous phosphate
Ferrous sulphate

Gelatine
Glucose
Glycerine

Hydrochloric acid
Hydrogen peroxide

Indigo Carmine
Iodine

Lactic acid
Litmus

Magenta, basic
Magnesium
Magnesium carbonate
Magnesium chloride
Magnesium sulphate
Malt extract
Maltose
Mannitol = Mannite
Mannite
Mercuric chloride
Mercury
Methyl alcohol
Methylene blue
Methyl orange
Methyl red

α-Naphthol
α-Naphthylamine
Neutral red
Nile blue
Nitric acid
p-Nitrobenzene azo-resorcinol

Orcein
Oxalic acid

Paranitrophenol
Paraphenylenediamine
Peptone
Petroleum ether B.T. 50–60° C.
Phenylhydrazin hydrochloride
Phenolphthalein
Phloroglucin
Potassium carbonate
Potassium chlorate
Potassium chloride
Potassium dichromate
Potassium ferrocyanide
Potassium hydroxide
Potassium iodide
Potassium nitrate
Potassium permanganate
Potassium phosphate (three types)
Potassium silicate
Potassium sodium tartrate
Potassium sulphate
Potassium thiocyanate
Pyrogallol

Resorcinol
Resorcinol yellow = Tropæolin O
Ruthenic chloride = Ruthenium red

Scharlach red = Sudan IV
Silver nitrate
Sodium acetate
Sodium amalgam
Sodium asparaginate
Sodium bicarbonate
Sodium bisulphite
Sodium bromide
Sodium carbonate
Sodium chloride
Sodium citrate
Sodium diborate
Sodium hydroxide
Sodium nitroprusside
Sodium phosphate
Sodium silicate
Sodium sulphate
Sodium sulphite
Sodium thiosulphate
Starch
Sucrose
Sudan III and IV
Sulphanilic acid
Sulphuric acid

Tartaric acid

Thymolphthalein
Tropæolin OO

Waterglass = sodium silicate

Zinc
Zinc chloride
Zinc sulphate

APPENDIX III

LIST OF CHEMICALS FOR PHOTOGRAPHIC WORK

Acetic acid
Albumen
Alcohol
Aluminium potassium sulphate
Amidol
Ammonium carbonate
Ammonium hydroxide
Ammonium persulphate
Ammonium sulphocyanide (thiocyanate)

Borax = sodium diborate
Boric acid

Citric acid
Copper sulphate

Ferrous sulphate
Formalin

Gold chloride

Hydrochloric acid
Hydroquinone
Hypo = sodium thiosulphate

Lead acetate
Lead nitrate

Mercuric chloride
Metol

Nitric acid

Pinachrome
Pinacryptol green
Pinacyanol
Potassium bromide
Potassium carbonate
Potassium dichromate
Potassium ferricyanide
Potassium iodide
Potassium metabisulphite
Potassium permanganate
Pyrogallol

Silver nitrate
Sodium acetate
Sodium bisulphite
Sodium carbonate
Sodium diborate
Sodium hydroxide
Sodium sulphate
Sodium sulphide
Sodium sulphite
Sodium thiosulphate
Sulphuric acid

Thiocarbamide

APPENDIX IV

ADDRESSES OF SCIENTIFIC SUPPLIERS

General Suppliers of Apparatus, Glassware, etc.

Baird & Tatlock (London), Ltd., 14–17 St. Cross Street, Hatton Garden, London, E.C. 1.

Standley Belcher & Mason, Ltd., Church Street, Birmingham.

Central Scientific Company, c/o W. Edwards & Co., Ltd., Allendale Works, Southwell Road, London, S.E. 5.

Flatters & Garnett, Ltd., 309 London Road, Manchester, 13.

A. Gallenkamp & Co., Ltd., 17–29 Sun Street, Finsbury Square, London, E.C. 2.

W. & J. George, Ltd., 157 Great Charles Street, Birmingham, 3.

Griffin & Tatlock, Ltd., Kemble Street, Kingsway, London, W.C. 2.

Philip Harris & Co. (1913), Ltd., 144–146 Edmund Street, Birmingham.

Wood Bros. Glass Co., Ltd., Barnsley.

Microscopes, etc.

Bausch & Lomb Optical Co., Ltd., Africa House, Kingsway, London, W.C. 2.

Charles Baker, 244 High Holborn, London, W.C. 1.

R. & J. Beck, Ltd., 69 Mortimer Street, London, W.C. 1.

Newton & Co., Ltd., 72 Wigmore Street, London, W. 1.

W. R. Prior & Co., 9–11 Eagle Street, Southampton Row, London, W.C. 1.

Spencer Lens Co., c/o Hawksley & Sons, Ltd., 17 New Cavendish Street, London, W. 1.

James Swift & Son, Ltd., 81 Tottenham Court Rd., London, W. 1.

W. Watson & Sons, Ltd., 14 Hadley Grove, High Barnet, Herts.

Incubators and Ovens, etc.

Charles Hearson & Co., Ltd., 68 Willow Walk, Bermondsey, London, S.E. 1.

Stains and Dyes (in addition to the above)

George T. Gurr, 136 New King's Road, Fulham, London, S.W. 6.

Vector Manufacturing Co., Ltd., c/o Baird & Tatlock (London), Ltd., 14–17 St. Cross Street, Hatton Garden, London, E.C. 1.

Chemicals (in addition to the above)

Boots Pure Drug Co., Ltd., Nottingham.

British Drug Houses, Ltd., Graham Street, City Road, London, N. 1.

Burroughs Wellcome & Co., Snow Hill Buildings, London, E.C. 1.

General Chemical and Pharmaceutical Co., Ltd., Judex Works, Sudbury, Middlesex.

Hopkins & Williams, Ltd., 16 St. Cross Street, Hatton Garden, London, E.C. 1.

Botanical Material Suppliers

Biological Supply Association, Rhydyfelin, Aberystwyth, Wales.

George H. Conant, Ripon, Wisconsin, U.S.A.

General Biological Supply House, Inc., 761–763 East 69th Street, Chicago, Ill., U.S.A.

Photographic Suppliers

Ensign, Ltd., 88 High Holborn, London, W.C. 1.

Jonathan Fallowfield, Ltd., 61–62 Newman Street, Oxford Street, London, W. 1.

Ilford, Ltd., Ilford, Essex.

Kodak, Ltd., Kodak House, Kingsway, London, W.C. 2.

Westminster Photographic Exchange, Ltd., 111 Oxford Street, London, W. 1.

NOTES ON THE PREPARATION OF SOLUTIONS

1. Where a number of alternative, but similar, receipts are given they have been numbered in consecutive order.
2. Unless otherwise stated the conc." or concentrated liquid is indicated in the case of acids.
3. Where the term " alcohol " is used ethyl alcohol is implied, and where a definite percentage is not given absolute alcohol should be used.
4. With solids the weights given include any water of crystallization, unless the anhydrous salt is specified.
5. Pure or " Analar " chemicals should be employed in making up solutions, especially those for microchemical tests; for photographic work less pure chemicals may sometimes be safely used.
6. Where the weights do not include decimals a high degree of accuracy in weighing is generally unnecessary.
7. Certain solutions do not keep well, and large quantities should not as a rule be made up. The filling up of bottles containing a small residue is to be deprecated; they should be well washed and refilled with the fresh solution. A small bottle full keeps better than a large bottle nearly empty.
8. It is desirable, in some cases, to grease the stoppers of bottles, especially those containing strong alkalis, to prevent their sticking. Stoppers generally yield to the careful application of heat if the liquid is allowed to come into contact with the stopper during the process.
9. Certain stains, such as hæmatoxylin, require a period of " ripening " before use. Others, such as magdala red, fade if exposed to bright light. Solutions of osmic acid should always be kept in the dark.
10. " Dropping bottles " should be used for all microscope bench reagents.
11. Always label bottles of solutions immediately they are made up. Time will often be saved if the receipt for the solution is written on the label. Labels on bottles of stains or of corrosive liquids should be protected by covering with a coat of melted paraffin wax.

	0	1	2	3	4	5	6	7	8	9	1 2 3	4 5 6	7 8 9
10	0000	0043	0086	0128	0170						5 9 13	17 21 26	30 34 38
						0212	0253	0294	0334	0374	4 8 12	16 20 24	28 32 36
11	0414	0453	0492	0531	0569						4 8 12	16 20 23	27 31 35
						0607	0645	0682	0719	0755	4 7 11	15 18 22	26 29 33
12	0792	0828	0864	0899	0934						3 7 11	14 18 21	25 28 32
						0969	1004	1038	1072	1106	3 7 10	14 17 20	24 27 31
13	1139	1173	1206	1239	1271						3 6 10	13 16 19	23 26 29
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						1614	1644	1673	1703	1732	3 6 9	12 14 17	20 23 26
15	1761	1790	1818	1847	1875						3 6 9	11 14 17	20 23 26
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16	2041	2068	2095	2122	2148						3 6 8	11 14 16	19 22 24
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19	2788	2810	2833	2856	2878						2 4 7	9 11 13	16 18 20
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20	3010	3032	3054	3075	3096	3118	3139	3160	3181	3201	2 4 6	8 11 13	15 17 19
21	3222	3243	3263	3284	3304	3324	3345	3365	3385	3404	2 4 6	8 10 12	14 16 18
22	3424	3444	3464	3483	3502	3522	3541	3560	3579	3598	2 4 6	8 10 12	14 15 17
23	3617	3636	3655	3674	3692	3711	3729	3747	3766	3784	2 4 6	7 9 11	13 15 17
24	3802	3820	3838	3856	3874	3892	3909	3927	3945	3962	2 4 5	7 9 11	12 14 16
25	3979	3997	4014	4031	4048	4065	4082	4099	4116	4133	2 3 5	7 9 10	12 14 15
26	4150	4166	4183	4200	4216	4232	4249	4265	4281	4298	2 3 5	7 8 10	11 13 15
27	4314	4330	4346	4362	4378	4393	4409	4425	4440	4456	2 3 5	6 8 9	11 13 14
28	4472	4487	4502	4518	4533	4548	4564	4579	4594	4609	2 3 5	6 8 9	11 12 14
29	4624	4639	4654	4669	4683	4698	4713	4728	4742	4757	1 3 4	6 7 9	10 12 13
30	4771	4786	4800	4814	4829	4843	4857	4871	4886	4900	1 3 4	6 7 9	10 11 13
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36	5563	5575	5587	5599	5611	5623	5635	5647	5658	5670	1 2 4	5 6 7	8 10 11
37	5682	5694	5705	5717	5729	5740	5752	5763	5775	5786	1 2 3	5 6 7	8 9 10
38	5798	5809	5821	5832	5843	5855	5866	5877	5888	5899	1 2 3	5 6 7	8 9 10
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47	6721	6730	6739	6749	6758	6767	6776	6785	6794	6803	1 2 3	4 5 5	6 7 8
48	6812	6821	6830	6839	6848	6857	6866	6875	6884	6893	1 2 3	4 4 5	6 7 8
49	6902	6911	6920	6928	6937	6946	6955	6964	6972	6981	1 2 3	4 4 5	6 7 8

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54	7324	7332	7340	7348	7356	7364	7372	7380	7388	7396	1 2 2	3 4 5	6 6 7
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56	7482	7490	7497	7505	7513	7520	7528	7536	7543	7551	1 2 2	3 4 5	5 6 7
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60	7782	7789	7796	7803	7810	7818	7825	7832	7839	7846	1 1 2	3 4 4	5 6 6
61	7853	7860	7868	7875	7882	7889	7896	7903	7910	7917	1 1 2	3 4 4	5 6 6
62	7924	7931	7938	7945	7952	7959	7966	7973	7980	7987	1 1 2	3 3 4	5 6 6
63	7993	8000	8007	8014	8021	8028	8035	8041	8048	8055	1 1 2	3 3 4	5 5 6
64	8062	8069	8075	8082	8089	8096	8102	8109	8116	8122	1 1 2	3 3 4	5 5 6
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66	8195	8202	8209	8215	8222	8228	8235	8241	8248	8254	1 1 2	3 3 4	5 5 6
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73	8633	8639	8645	8651	8657	8663	8669	8675	8681	8686	1 1 2	2 3 4	4 5 5
74	8692	8698	8704	8710	8716	8722	8727	8733	8739	8745	1 1 2	2 3 4	4 5 5
75	8751	8756	8762	8768	8774	8779	8785	8791	8797	8802	1 1 2	2 3 3	4 5 5
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82	9138	9143	9149	9154	9159	9165	9170	9175	9180	9186	1 1 2	2 3 3	4 4 5
83	9191	9196	9201	9206	9212	9217	9222	9227	9232	9238	1 1 2	2 3 3	4 4 5
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89	9494	9499	9504	9509	9513	9518	9523	9528	9533	9538	0 1 1	2 2 3	3 4 4
90	9542	9547	9552	9557	9562	9566	9571	9576	9581	9586	0 1 1	2 2 3	3 4 4
91	9590	9595	9600	9605	9609	9614	9619	9624	9628	9633	0 1 1	2 2 3	3 4 4
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97	9868	9872	9877	9881	9886	9890	9894	9899	9903	9908	0 1 1	2 2 3	3 4 4
98	9912	9917	9921	9926	9930	9934	9939	9943	9948	9952	0 1 1	2 2 3	3 4 4
99	9956	9961	9965	9969	9974	9978	9983	9987	9991	9996	0 1 1	2 2 3	3 3 4

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